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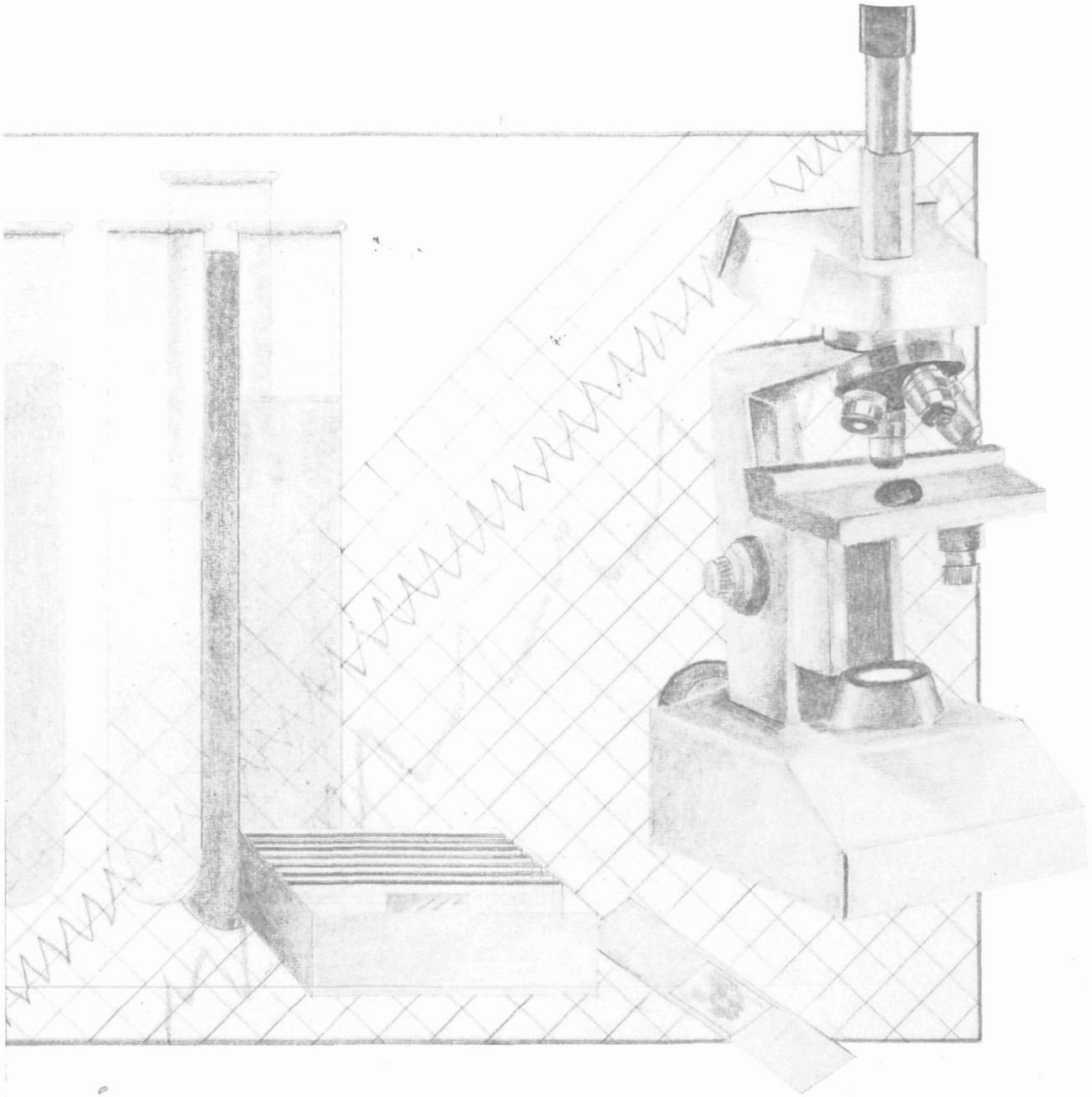
Hazard Evaluation Division Standard Evaluation Procedure

Oncogenicity Potential (Guidance for Analysis and Evaluation of Long Term Rodent Studies)



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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

ONCOGENICITY POTENTIAL:

GUIDANCE FOR ANALYSIS AND EVALUATION OF LONG TERM RODENT STUDIES

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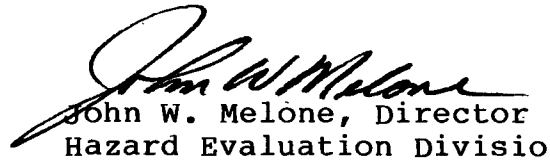
This temporary revision is to be used until public comments on the EPA Proposed Guidelines for Carcinogen Risk Assessment (2) are evaluated and are adopted in final form.

STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.



John W. Melone, Director
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Preamble

For government agencies concerned with public health and/or environmental effects, one of the most difficult tasks is the identification and regulation of potential (suspect human) oncogenic substances, be they food additives, drugs, pesticides or industrial chemicals. In the Environmental Protection Agency, the regulatory process is based on scientific evidence, and socioeconomic considerations. Within EPA, the Office of Pesticides and Toxic Substances (OPTS) is responsible for the evaluation of the scientific evidence relative to health risk assessments for pesticides and industrial chemicals.

The most persuasive evidence of potential oncogenicity in man comes from competently designed and conducted human epidemiology studies supported by appropriate animal studies. However, the most frequently seen evidence is based on long-term tests in laboratory animals such as mice and rats. *In vivo* and *in vitro* short-term studies (e.g., mutagenicity), biochemical reactivity information, and metabolic and pharmacodynamic studies provide additional and sometimes critical evidence.

This document may not contain anything new or revolutionary regarding the evaluation of oncogenic potential evidenced by toxic substances. However, it is the first time, as far as I know, that the weight of scientific evidence concept, as being developed by OPTS, and major considerations for analysis

of animal oncogenicity data have been brought together in one place. This document treats complex issues, incompletely and in some cases superficially. However, it represents a base from which OPTS might further develop and strengthen its hazard identification and risk evaluation abilities in this complex area.

It is possible that evaluation of the strength of biological and auxiliary evidence for oncogenicity, prior to the mathematical calculation of risk, will prevent the untimely polarization of opinion and interject a higher degree of understanding and confidence into the hazard identification and risk assessment process. It is also possible that the standardization of data organization and evaluation procedures might increase the efficiency, thoroughness and consistency with which individual evaluations are prepared and help the evaluators reach considered and scientifically defensible judgments. To this end, all references are intended to be an integral part of the guidance offered by this document and they should be read.

It must be understood by evaluators that their major responsibility is the competent analysis, evaluation, and interpretation of biological and toxicological data according to sound scientific principles. Therefore, evaluators must not

allow their deliberations to be overly influenced by the ambiguities of controversial concepts or by their perception of what potential regulatory decisions or actions their evaluations may portend. The latter pitfall may be ameliorated by recognizing that the scientific identification and assessment of risk is a separate and distinct function from risk management [i.e., regulatory decision making].[1]

Three things are certain, no matter how well these guides are followed: 1) they will not automatically produce a "carcinogenic" vs. "non-carcinogenic" decision; 2) substitute for sound scientific judgment; nor 3) prevent criticism or controversy regarding the judgments made and the conclusions drawn. However, a review which exhibits internal evidence of: a) being based on sound scientific principles; b) presenting succinct and cogent rationale for judgments and conclusions; c) presenting quoted material, (i.e., text or tabular) with proper citation; and d) having been competently and objectively performed, will require critics to focus their arguments with equal competence, completeness, and succinctness.

As more experience in use of the weight-of-evidence procedure is obtained, contents of this document should be modified and improved. To this end all recommendations will be most welcome. I am very indebted to my colleagues who have previously performed this valuable service.

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8/9/84

I. Analysis for Oncogenic Potential in Experimental Animals

A. Introduction

The Environmental Protection Agency (EPA) has published a request for public comment on Proposed Guidelines for Carcinogen Risk Assessment.[2] The purpose of the proposal is to incorporate the concepts and approaches to oncogenic risk assessment which have been developed since the 1976 Interim Procedures and Guidelines were issued.[3] Although the 1976 guidelines will be eventually superceded, they are briefly discussed here because they provide a very clear picture of the EPA regulatory process as well as an informative statement of the weight-of-evidence concept.

The 1976 guidelines described the decision process regarding the regulation of potential oncogens as being two-phased. The first phase is the determination that a particular substance constitutes an oncogenic risk. The second phase is the determination of what regulatory action, if any, should be taken to reduce that risk. Accordingly, they state: "The central purpose of the health risk assessment is to provide a judgment concerning the weight-of-evidence that an agent is a potential human carcinogen and, if so, how great an impact it is likely to have on public health" [underlining added].[4]

In addition the guidelines leave no doubt that an analysis of health risks must be separate and independent from any consideration of the socioeconomic consequences of regulatory action. This is also true of the proposed guidelines.

The preamble of the 1976 guidelines clarifies the meaning of the "weight-of-evidence" concept.

> In considering the risks, it will be necessary to view the evidence of carcinogenicity in terms of a warning signal, the strength of which is a function of many factors including those relating to the quality and scope of the data, the character of the toxicological response, and the possible impact on public health. It is understood that qualifications relating to the strength of the evidence for carcinogenicity may be relevant to this consideration because of the uncertainties in our knowledge of the qualitative and quantitative similarities of human and animal responses. In all events, it is essential in making decisions about suspect carcinogens that all relevant information be taken into consideration.[5]

The weight of biological evidence concept used in this Evaluation Procedure is that part of the assessment process which considers and weighs the cumulative observational and experimental data pertinent to arriving at a level of concern about a substance's oncogenic potential for humans. It is

composed of a series of judgments concerning the adequacy, validity, and appropriateness of the observational and experimental methods used to produce the data base and, those judgments which bring into causal, complementary, parallel, or reciprocal relationships all the data considered. Because our knowledge concerning oncogenic mechanisms is still developing, because good epidemiological evidence is seldom available and because animal studies are not always conclusive, all of the information available at a given time may provide only "persuasive evidence" (i.e., not clearly robust or feeble, yet suggestive of a defensible presumption) one way or the other about the human oncogenic potential of a given substance. It is for this reason that both guidelines stress the importance of succinctly articulating the rationale for judgments and conclusions contained in risk assessment and the uncertainties pertaining thereto. This becomes important when new data or new scientific knowledge requires reevaluation of the data base or a change in a previous risk assessment or regulatory action.

The 1984 Proposed Guidelines describe in general terms, the essential aspects of risk assessment and present salient principles which are the foundation for evaluating biological and other types of data relating to suspect carcinogens. They embrace the scientific principles of carcinogenic assessment developed by the Office of Science and Technology

Policy [OSTP][6] and with modifications, the concept of risk assessment developed by the National Research Council [NRC][1] and the weight-of-evidence scheme developed by the the International Agency for Research on Cancer [IARC].[7]

The Proposed Guidelines describe the method to be used for evaluating studies thus:

> Studies are evaluated according to sound biological and statistical considerations and procedures. These have been described in several publications [(6) and (8) through (17)]. Results and conclusions concerning the agent, derived from different types of information, whether indicating positive or negative responses, are melded together into a weight-of-evidence determination. The strength of the evidence supporting a potential human carcinogenicity judgment is developed in a weight-of-evidence stratification scheme.

Pertinent parts of the weight-of-evidence stratification scheme which is an adaptation of the IARC approach to EPA needs, is presented in Part II of this document. This scheme and the guidance provided by the references cited in the above quote must be used within the Agency for all oncogenicity study evaluations. This will assure the desirable uniformity of procedures and conformity to the Agency's prescribed philosophy for analysis, evaluation, interpretation, and

classification of data generated by such studies. For these reasons it behooves every evaluator to understand, and strictly adhere to the guidance presented in the Proposed Guidelines and the cited references. Any discrepancy between the general approach presented by the 1984 Proposed Guidelines and the guidance offered in this Evaluation Procedure must always be reconciled in favor of the Proposed Guidelines.

The NRC describes risk assessment as containing some or all of the following steps or components: 1) hazard identification; 2) dose-response assessment; 3) exposure assessment; and 4) risk characterization.[1] Guidance offered by this Evaluation Procedure is confined entirely to the NRC hazard identification component. This is in keeping with the Proposed Guidelines which place the other three components within the dominion and under the aegis of scientists skilled in the quantitative aspects of health risk assessments. This is clear from the following Proposed Guideline discussion of the four components [also see Part III. A. 1. of Reference 2]:

> Hazard identification is a qualitative risk assessment, dealing with the process of determining whether exposure to an agent has the potential to increase the incidence of cancer. For purposes of these guidelines, malignant and benign tumors are used in the evaluation

of the carcinogenic hazard. The hazard identification component qualitatively answers the question of how likely an agent is to be a human carcinogen.

The dose-response assessment defines the relationship between the dose of an agent and the probability of induction of a carcinogenic effect. This component usually entails an extrapolation from the generally high doses administered to experimental animals or exposures noted in epidemiological studies to the exposure levels expected from human contact with the agent in the environment; it also includes consideration of the validity of these extrapolations.

The exposure assessment identifies populations exposed to the agent, describes their composition and size, and presents the types, magnitudes, frequencies, and duration of exposure to the agent.

In risk characterization the output of the exposure assessment and the dose-response assessment are combined to estimate quantitatively some measure of the carcinogenic risk. As part of risk characterization, a summary of the strengths and

> weaknesses in the hazard identification, dose-response assessment, exposure assessment, and the public health risk estimates are presented. Major assumptions, scientific judgments, and, to the extent possible, estimates of the uncertainties embodied in the assessment are also presented, distinguishing clearly between fact, assumption and science policy.

For discussion of the elements of hazard identification and the types of data which are relevant to this component see Parts II, B. 1. through 7. of the Proposed Guidelines.[2]

In keeping with the Proposed Guidelines and to bring a sharper focus on the analysis and evaluation of experimental animal data, OPTS adopts the following definitions and major considerations as part of its oncogenic hazard identification procedures.

B. Definition of Chemical Oncogenicity.

The International Agency for Research on Cancer (IARC) has developed the following widely accepted meaning of the term chemical carcinogenesis.

> Chemical carcinogenesis...is [a] the induction by chemicals of neoplasms that are not usually observed, [b] the earlier induction by chemicals of neoplasms that are commonly observed, and/or [c] the induction by chemicals of more neoplasms than are usually found--although fundamentally different mechanisms may be involved in these three situations.[18]

OPTS has adopted this meaning as its working definition with one exception. The term "oncogenicity" is substituted for the term "carcinogenicity." Carcinogenicity, etymologically, means induction of malignant neoplasms. The above definition does not make a distinction between benign and malignant neoplasms and OPTS should not do so in a working definition. In the evaluation of health risk, the nature and incidence of all types of neoplasms and their possible interrelationships should be considered. Therefore, the term "oncogenicity" is deemed more appropriate.

For the sake of clarity, some implications in this definition need to be made explicit. All sections of the IARC definition, imply that the evaluator has knowledge of the types of neoplasms "usually or commonly observed" in the animals used in the study being evaluated, including knowledge that goes beyond the information derived from the concurrent control animals for a particular study or group of studies (i.e., historical control data). Part II. B.6. of the Proposed Guidelines also assumes familiarity with relevant historical control data. Situation (a) of the IARC definition implies that the reviewer has knowledge of those neoplasms which are not usually observed or found, i.e., rare or unusual neoplasms. Situation (b) implies that OPTS should obtain or derive, and use, data which bears on the time of tumor appearance (decreased latency or precocity of onset) and will know when each neoplasm type is usually expected to appear or be discovered in the animals used. Finally, the definition implies that OPTS should try to identify data (e.g., mutagenicity, metabolism, and biochemical reactivity data) suggestive of mechanisms which may be involved in the neoplasia produced by the specific chemical.

The Proposed Guidelines relating to hazard identification, the above working definition and its implications, and the collective experience of OPTS are the foundation for development of the following considerations.

C. Documentation and Data Acceptance

The quality, integrity, and completeness of reporting observational and experimental data are essential to the proper analysis and evaluation of study results. In essence, the "good science" evaluations expected of OPTS have their foundations in the evaluated evidential documentation. Therefore, qualitative evaluation of the documentation of a particular study or group of studies is of special significance to the acceptability of data.

The following three important considerations address the acceptability of long-term rodent studies.

1. The adequacy of the experimental design and other experimental parameters such as: the route of administration; frequency and duration of exposure; appropriateness of the species, strain, sex, and age of the animals used; choice of dosage levels; appropriateness of the observational and experimental methods; and the conditions under which the substance was tested.

There are no specific, internationally agreed upon scientific rules or fixed check lists which make the judgment regarding the acceptability of data bases a standard routine procedure. However, there are suggested guidelines concerning the mechanics of good experimental design, reporting, and good laboratory practice which are aids to evaluation of

report and data acceptability. These may be found in the EPA suggested guidelines and the EPA and FDA Good Laboratory Practices Regulations. The Proposal Guidelines[2] state that criteria for the technical adequacy of animal studies can be found in prescribed publications[6, 10, 14, 15, 16, 17, 19, 20, 21] and that these should be used to judge the acceptability of studies. The evaluator needs to be cautious when using the above guidance as aids to making an acceptability judgment for any oncogenicity study. The cardinal question to be answered is how well does the study, in toto, facilitate the identification of a potential oncogenic effect or lack thereof for the substance being evaluated, and not how precisely does the study fit a prescribed recipe for performance. The collective experience of OPTS scientists can be very helpful in resolving difficult questions of acceptability and should be utilized whenever it is needed.

As the first step in the evaluation process, the evaluator should carefully read through the report including supporting data presentations, and make a tentative classification of acceptability prior to making a detailed evaluation of the individual data. If there are obvious and significant deficiencies in the report, any further work would be a waste of resources. The submitter of the report should be notified of the problem(s) as quickly and as accurately as possible and any further analysis suspended until these deficiencies are corrected.

Frequently, the subsequent detailed analysis of the data will bring to light deficiencies which were not obvious during the initial reading of the report. In this case the deficiencies should be noted and the evaluation completed as far as possible. At this time the submitter of the document should be made aware of the situation along with any scientific questions or other data needs identified during the detailed data analysis and evaluation.

2. The competency and completeness with which the study or studies were conducted and reported.

Doubts on the part of an evaluator regarding completeness and/or competency with which a study was performed or reported must be discussed with the evaluator's supervisor. If these concerns are judged to be reasonable at this level, the study should be nominated for a laboratory and data base audit. Any further consideration of the study should be suspended until the audit is completed and the results evaluated.

3. The effects of modifying factors such as differential survival, toxicity, or disease which result in major inequalities between control and treated animals.

This qualitative consideration has more to do with the evaluation and interpretation of data than with the

acceptability of documentation. However, it is placed here because determination of the various factors influencing toxicological and oncological data, as may be indicated in the evidential documentation, needs to be made prior to application of the major oncogenicity conderations.

There are many factors influencing the response of experimental animals to chemical substances. Some of these are discussed by Doull[22] and his presentation of this subject should be reviewed. Of special interest in oncogenicity data evaluation are the factors contained in this qualitative consideration.

Differential survival in any animal group, regardless of its cause, has an important bearing on the evaluation and interpretation of oncogenicity studies. An apparent unequal reduction, real or illusory, of the number of animals at risk in oncogenicity studies is a complicating influence and may lead to serious misinterpretation of a substance's oncogenic potential. Therefore, it is essential to determine, early in the review period, if this factor has any significance for the proper application of the major oncogenicity considerations. This determination may not always be a simple routine [for an example, see reference 79] and the services of a competent statistician should be obtained in the case of doubt or controversy. Because of the importance of this factor, time

to death or sacrifice, preferably in days, of each animal should be presented by or obtained from the report submitter. Such information is useful in certain statistical procedures (e.g., lifetable method) and may be useful in evaluation of time to tumor data (see major consideration # 6).

As with differential survival/mortality, the presence of toxic or pharmacological effects or disease processes can complicate the evaluation and interpretation of data and, depending on severity, can cause the study to be of very limited value for evaluation of the oncogenic potential of a substance. The effects of these factors can be particularly troublesome when they are confused with or misinterpreted as preneoplastic lesions. Examples of these types of problems can be found in references 80 and 81. Problems related to the above factors should be resolved prior to application of the major oncogenicity considerations.

The three qualitative considerations for documentation and data acceptance discussed above are applicable to all experimental animal studies, no matter what their intended purpose might be, and essentially establish the acceptability not only of specific reports but also the acceptability of the eventual evaluation, interpretation and judgments based upon them.

Resolution of problems related to qualitative or quantitative considerations is not entirely the responsibility of the individual evaluator. The submitter of the evidential documentation may be requested to assist. For particularly difficult problems, the assistance of consultants and the Scientific Advisory Panel for pesticides and the Science Advisory Board for other chemicals may be utilized. Requests for the latter types of assistance must be through OPTS management.

The acceptability of reports and other technical information submitted to OPTS is a scientific judgment and only secondarily a legal one. Therefore, OPTS bears the burden of defending and documenting the acceptance or rejection, in part or in whole, of the evidential documentation and data. The submitters of information deserve to know the rationale for any rejection of data. This rationale should be succinctly stated in the evaluation document.

D. Major Considerations for Analysis and Evaluation of Oncogenicity

1. Spontaneous neoplasm incidence in untreated animals (concurrent and historical controls).

It is well known among experienced pathologists and toxicologists that the incidence of spontaneous lesions, including neoplasms, is unpredictably variable among groups of concurrent controls in the same study as well as among control groups of the same strain from different studies and laboratories.[23] Tables 1 and 2 present observed examples of both situations. Such variation is frequently encountered and oftentimes complicates the evaluation and interpretation of toxicity studies in general and oncogenicity studies in particular. Some of the difficulty in interpretation can frequently be ameliorated by the judicious consideration of historical control (spontaneous incidence) data. Such data should be viewed as an auxiliary aid to interpretation of study data. It should not be used as a complete substitution for concurrent control data within a particular study or group of studies.

The Task Force of Past Presidents of the Society of Toxicology gives the following examples of how historical data may be useful.

The following propositions may be taken as scientifically useful in the evaluation of a chemical carcinogenic response, with distinctions drawn between the use of concurrent control and historical control data. (1) If the incidence rate in the concurrent control group is lower than in the historical control groups, but the incidence rates in the treated groups are within the historical control range, the differences between treated and control groups are not biologically significant. (2) If the incidence rates in the treated groups are higher than the historical control range but not statistically significantly greater than the concurrent control incidence, the conclusion would be that there is no relation to treatment, but with the reservation that this result could be a false negative resulting from some flaw. (3) If the incidence rates in the treated groups are significantly greater than in the concurrent controls, and greater than the historical control range, a treatment effect may be present which is unlikely to be a false positive test.[24]

Table 3 presents two actual examples of how the National Cancer Institute (NCI) has used spontaneous incidence data (historical control data) as an aid to interpretation of treatment relationships of particular study lesions. These examples were chosen because they approximate situations 1) and 2) presented above by the Task Force of Past Presidents. Note that historical control data was not substituted for concurrent control data in either of these examples.

The best historical control data are obtained using the same species and strain, from the same supplier, maintained under the same general conditions in the same laboratory which generated the study data being evaluated. The data should be from control animals on recent, (5 years but no later than 10 years) consecutive, long-term oncogenicity studies. However, even this type of data can be misleading if not properly organized, evaluated, and interpreted.

It is highly desirable to obtain data from individual groups of control animals, in order to establish a range of values, rather than from combined groups of animals yielding only a single mean value. On this matter, the Task Force of Past Presidents presented the following examples as a word of caution:

Historical data are often presented as the incidence of tumor in hundreds of control animals. Statistical procedures can be used to relate this overall incidence to the incidence in a specific study. However, this leaves much to be desired since the incidence of tumors can vary considerably between groups of animals. Thus, in 11 carcinogenic studies in rats (Charles River Caesarian Derived) [Sprague Dawley] where there are two or three concurrent control groups of animals, the incidence of brain tumors varied from 1% to 10% in male rats in three concurrent control groups. The female rats had no brain tumors. In other control groups of male rats, the incidence of brain tumors varied from 0 to 4%. This type of variation is not apparent if the incidence in combined control animals is used.

In another example, the overall incidence of pheochromocytomas in 1,100 control male rats was 2% but the variation among groups was 0 to 28%. In one of these studies, the incidence of pheochromocytoma in male rats was 8% in one control group, 28% in the concurrent second

> control group and 14, 18 and 26% in the low, middle and high dose groups of treated animals, respectively. If only the control group with 8% incidence of pheochromocytomas had been used, there would have been a significant difference between the control group and the high dose group, and the presence of an upward trend would have resulted in the conclusion that the chemical was a carcinogen for male rats. Obviously, this was not the case.[24]

It is also necessary to be cautious concerning what is really represented by tabulated incidence data, spontaneous incidences or otherwise. Sometimes investigators combine certain types of neoplasms when presenting tumor incidences in summary tables. Usually a careful reading of the text accompanying the summary data table will indicate where tumor combination has occurred.

To avoid entrapment in these types of pitfalls, evaluators should specifically request that historical control data be presented for each neoplasm as <u>discrete control group incidences, segregated by sex, and updated with each new study submission</u>.

It is also highly desirable that additional information on each discrete control group be made available. This information should include the following:

a. Identification of species and strain and name of the supplier including specific colony identification if supplier has more than one geographical location.

b. Name of the laboratory in which the study was performed, and when;

c. Description of general conditions under which the animals were maintained, including the type or brand of diet, and type of bedding if possible;

d. The planned duration of the study (e.g., 18 months or 2 years) and the approximate age, in days, of the control animals at the beginning of the study and at the time of killing or death;

e. Description of the control group mortality pattern observed during or at the end of the study and of any other pertinent observations (e.g., diseases, infections, etc.);

f. Name of the pathology laboratory and examining pathologist responsible for gathering and interpreting the pathological data from the study; and

g. What tumors may have been combined to produce any of the incidence data.

Examples of how historical control data was used in resolving problems related to a disease incidence may be found in reference 81. An example of a contrary view of historical incidence (control) data use has been articulated.[82]

2. Presence and incidence of neoplasms not usually observed (rare or unusual neoplasms).

The terms "rare" and "unusual" when applied to scientific observations or events are generally understood to mean infrequently occurring or not ordinarily or commonly encountered. Thus the terms are used synonymously for purposes of applying this major oncogenicity consideration to the data base.

Statistical analysis of lesions observed with low frequency* in a particular study presents difficult methodological and interpretational problems and may be of extremely limited usefulness as an aid to judging the rarity of such lesions and their relationships to treatment. Therefore, the

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* For purposes of this discussion the criteria used by NCI--primary tumors occurring in two animals or less in one of the control or treated groups of 50 animals each and/or where such tumors are observed in less than 5% of the group,--defines low frequency.[25]

evaluator must seek another guidepost for the attribution of the term "rare" or "unusual" and treatment relationship to the lesions of low incidence observed in a specific study or group of studies. Historical control (spontaneous incidence) data are most useful in this situation. However there is one particularly important pitfall which must be recognized.

If the standards for applying these attributes to specific types of toxicologic or oncologic observations or lesions were static, there would be much less difficulty in the evaluative and interpretative processes. However, as the opportunity for observation increases with time or the development and use of more precise or sensitive methods of observation and detection, the rarity and unusualness of an event may remain relatively stable or may slowly or quite rapidly change.

One example of this phenomenon is presented by the 1963 work of Thompson and Hunt.[27] The authors decided to re-examine, "by serial section techniques, representative organs in which neoplasms, that were not grossly apparent, had been originally detected upon microscopic examination of randomly selected single tissue sections." The results of the two examinations are presented in Table 4.

The most impressive change in tumor incidence occurred in the thyroid light-cell (C-cell) adenoma. By use of a more precise technique, the combined observed incidence (all rats)

rose from 9/140 (6%) to 55/140 (39%). The incidence for males and females each rose about six-fold. Since the pathologists were the same, the increased incidence in C-cell adenomas can only be explained by the increased opportunity for observation provided by the serial sectioning technique. The authors concluded that spontaneous light-cell adenomas occur with about equal frequency in both sexes of the Sprague-Dawley strain and, (more importantly for the present illustration) "that this type of tumor is far more common in the rat than previous reports might suggest."

By contrast, in this study, the brain tumor incidence did not change because of the increased opportunity for observation. The incidence for all tumor types and for each type individually remained 4/126 (3.2%) and 1/126 (0.8%) respectively. From these data one could conclude that the observed frequency of brain tumors in the Sprague-Dawley rat is low and therefore they are not usually or commonly encountered (i.e., rare). This conclusion is supported by the 1973 work, a decade later, of MacKenzie and Garner.[28] These pathologists examined various tissues of six rat strains from different sources. Serial sectioning methods were not used. The results for brain tumors only are presented in Table 5.

In this study the incidence of brain tumors observed in the Sprague-Dawley rat was also 0.8% (2/258). The incidence of all brain tumors among the six strains was 17/2082 (also 0.8%) and ranged from 3/535 (0.6%) for the Charles River-SD

rats to 4/217 (1.8%) for the Diablo-SD rats. See reference 29 for further discussion of brain tumor incidence.

In the case of brain tumors in rats, the incidence has remained relatively stable with increased time and increased opportunity for observation. From such types of studies and their own experience pathologists have reached the consensus that brain tumors in rats might be considered for the present rare and unusual neoplasms. For this reason they require special attention during the evaluation process as do all other lesions exhibiting this attribute.

The knowledge that shifts in observed spontaneous incidences for some lesions does occur with increased opportunity for observation and increased sensitivity of detection should not be a major impediment to use of historical control data, especially if such data are continually updated.

References 83 and 84 provide examples of problems concerning rarity of a tumor or groups of tumors and how historical incidence (control) data were useful in their resolution.

3. Increased incidence of benign and/or malignant neoplasms that are usually found.

The pathologist has a unique position in toxicological and oncological evaluations. Evaluators are usually entirely dependent on such individuals for descriptions of the variety of spontaneous and treatment/disease induced lesions present at any time during a study. This is especially true regarding the distinction between benign and malignant neoplasms.

Zbinden, in discussing this subject, points out the special role of the pathologist in providing information on the morphology of lesions and emphasizes that these data will also establish the presence or absence of a dose-effect relationship for some of the lesions. This information is obviously critical to the establishment of toxic effects produced by the substance and its oncogenic potential. Zbinden briefly discusses the use of semi-quantitative methods as well as more accurate morphometric methods for rating the severity of lesions, but cautions that even with their use we can not be entirely satisfied with diagnostic labels for lesions because of the lack of generally and internationally accepted nomenclature in toxicological pathology. He gives the following interesting example of what could happen because the pathologist is permitted to coin his own diagnostic labels for a mammary gland nodule: 1) it can be labeled "cystic fibromatous hyperplasia" and make it sound innocent; 2) "ductal carcinoma in situ" to sound frightening; or 3) being noncommittal -mammary hyperplasia with squamous metaplasia and a certain potential for malignant (carcinomatous of sarcomatous) degeneration[30]

To prevent this type of problem, an experienced pathologist will describe each significant lesion type, at least once, in such detail that any competent pathologist can perceive a good mental picture of the lesion and form his own judgment as to its relevance to the histopathology induced by the chemical being tested. In spite of improvements in methodology and descriptive reporting, this area of highly subjective judgments often times presents special problems of quantification and reproducibility for toxicologists.

Further examples of potential problems caused by total reliance on diagnostic labels are provided in Table 6. Note that the term "hepatoma" has appeared in the scientific literature as a label for both benign and malignant neoplasms. Also the term "nodular hyperplasia" has been used as a label for benign neoplasms and for hyperplasia, in spite of the fact that the latter is a non-neoplastic lesion.

Most problems with diagnostic terms are encountered in incidence tables, basically because the tabular information is meant to summarize descriptive information. For example, if a table listing liver effects contained only the term "hepatoma" as the sole designator for tumors, an evaluator would not know if the incidence data designated benign or malignant tumors or a combination of both types. Conversely, if the table listed individual liver effect incidences for

nodular hyperplasia, adenomas, and hepatocellular carcinomas, the evaluator should understand that the pathologist has made a distinction concerning these different effects and tumor states. However, if the tabulation only lists nodular hyperplasia and hepatocellular carcinoma, the evaluator does not know whether the nodular hyperplasia should be placed in a hyperplastic or a metaplastic category.

Sometimes incidence tables will contain a collective diagnostic term as a convenient substitute for more cumbersome diagnostic terms which do not conveniently fit the tabular format (e.g., substitution of "adenomatosis", a term which can be used to label an inflammatory process or a preneoplastic lesion, in a table for "focal area of alveolar cell proliferation").

There are only two alternatives for ameliorating this type of confusion. The first is to rely on the pathologist's detailed description of the lesion contained in the evidential documentation. If, however, the submitting pathologist has not provided a suitable description of the types of lesions or neoplasms found in the study and/or stated his criteria for distinguishing between a benign and malignant neoplasm, he should be requested to do so before the evaluation is completed. The other alternative is to request the original tissue slides and have them examined by the OPTS pathologist(s) or a competent consultant. Either of these requests should be made through

the OPTS management.

For an example of interpretational perturbations caused by the "adenomatosis" substitution cited previously and how these were resolved by using the pathologist's description, see reference 85.

While a competent pathologist must be relied upon for a final decision regarding the benign or malignant status of a given neoplasm, a general knowledge of the characteristics of both types of neoplasms is useful to the evaluator in the analysis and interpretation of incidence tables. Some of these are presented in Table 7. These should be perused by all evaluators from time to time to prevent the possibility of inappropriately combining benign and malignant neoplasms during the analysis and evaluation of a study. An example of what can happen when these characteristics are ignored or misinterpreted can be found in reference 86.

The evaluator should also be aware of the differences which may exist between those neoplasms potentially related to treatment and those which are <u>not</u> so related (spontaneous neoplasms). Ward and Reznik (32) have discussed some of the differences, (Table 8). While this concept is not completely accepted by a majority of pathologists, these differences may be of aid to evaluation. However, if there are any doubts on the part of the evaluator about the relationship of neoplas-

tic lesions to treatment, an experienced pathologist should be consulted.

For this major oncogenicity consideration keeping in mind the above pitfalls, all neoplasms observed in one or more treated groups which, by inspection, appear to have an incidence approximately equal to or higher than the concurrent control incidence should be identified (low frequency tumors have been previously discussed). Such data is often displayed in the evidential documentation in two different forms approximating Tables 9 and 10. The first of these is a listing of lesions by individual animals in each group and is useful in determining the number of animals per group exhibiting each lesion type. The second is a summary incidence which presents the number of tissues examined per group exhibiting each lesion type. The data needed for this consideration can be obtained, for the most part, from the Summary Incidence Table (see Table 10) submitted with most long-term study reports, provided the summary data has been verified as to its accuracy. However, if appropriate for completeness of review or for other reasons, the incidence data may be rearranged and displayed in a more convenient form. If rearranged by the evaluator or a statistician the tumor incidences should be segregated by sex, dosage levels, and tissue or organ site. Part III. B. 1. of Reference 2 states in part:

> Benign tumors should generally be combined with malignant tumors for risk estimates unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same morphologic type. However, the contribution of the benign tumors to the total risk should be indicated.

In order to comply with this latter requirement the incidence data for benign and maligant neoplasms of the same histogenic origin found in the same site should be reported as separate incidences. If the data submitter also wishes to present combined incidence data, it should be done in a manner simulating Table 1 and 2.

The combination of benign and malignant tumors or tumor sites to evaluate biological and/or statistical significance is a controversial issue. It is frequently done and may influence incidence rates and thereby the weight-of-evidence for oncogenicity. The basis for the appropriateness or inappropriateness of combining tumor types and incidences is their histogenesis. Therefore, when in doubt evaluators should not combine tumor data without the advice of the OPTS pathologist. When the combination occurs in the evidential documentation, the evaluator should expect to find the rationale clearly stated. If the rationale is absent, it should be requested from the responsible pathologist or statistician.

The National Toxicology Program (NTP) has become involved with problems attending to the combining procedure and has proposed draft guidelines[33] to its Board of Scientific Counselors for consideration. These are not yet officially promulgated by NTP. The reference should be consulted for the rationale for their proposed use. The following quote and table are presented here as an illustration of present thinking on this subject.

> Following is a list [Table 11] of organs/tissues where combining benign and malignant tumors is or is not appropriate to obtain a clearer understanding for the evidence of carcinogenicity. This list comprises those organs/tissues in which neoplasia is most often observed in Fischer 344 rats and B6C3F1 mice and may or may not be appropriate for use in other strains/species. Entities not on the list would be addressed on a case-by-case basis; this is a guide only. In addition, as the depth of knowledge increases in regard to the biological behavior of neoplasms in a given organ/tissue, certain combinations in the future may become inappropriate or appropriate.[33]

Great care must be exercised when rearranging incidence data since failure to list all tumors or double listing

of tumor types in any animal group may change the biological and/or statistical significance of the collected data and lead to a specious conclusion concerning the weight-of-evidence. Special care must also be taken when the evaluator is confronted with evidence of widespread systemic disease processes, e.g., amyloidosis, arteriosclerosis, or neoplasms of the hematopoietic system. Evidence of such disease processes is usually present in multiple organs and tissues within the individual animals. These lesions should be counted only once in individual animals. Skin tumors of basal cell origin should be counted together regardless of their many synonyms. Advice of the OPTS pathologist should be obtained if there is any doubt on the part of the reviewer as to how to derive an accurate incidence for systemic hematopoietic disease and skin tumor lesions.

Examples of problems created by inappropriate combination of tumor types have already been presented.[85, 86]

4. Degree of Induced Oncogenicity.

For purposes of this consideration, "degree" is defined as the relative amount of a quality, attribute, or condition and "induced" means stimulated an occurrence, or caused.

For this consideration, the evaluator should first determine if there is a pattern of potentially induced oncogenicity discernible in a study or group of studies. Occasionally, a study will exhibit a high degree of oncogenicity in all animal groups. The evaluator may obtain an impression of this situation and its severity by examining the total number of animals/sex/group exhibiting neoplasms of any kind during the course of the study. Such data can usually be derived from study tables presenting individual animal lesions (i.e., Table 9).

Table 12 presents data recently encountered at termination of a two-year rat study. This situation is an untidy complication to say the least. On the surface, the high degree of oncogenicity in all animal groups obscures any meaningful pattern of neoplasia or any potential dose response relationships. It also would appear that the degree of neoplasia in all groups is severe enough to place the study in the questionable category regarding its usefulness for evaluating oncogenicity. If a study exhibiting this degree of oncogenicity is the only data available or if it represents one-half of the long-term data base, the problem is a serious one and the study may have to be repeated.

However, before rejecting the study as unsatisfactory, further analysis should be done. In the situation above, a subsequent identification of the predominant types of neoplasms

and the degree of malignancy exhibited by all groups revealed that two tumor types, pituitary and mammary gland tumors, accounted for the high overall incidence and that increased incidence of malignancy was not a significant factor in any group [see reference 87 for details of analysis]. Since the study represented 1/6 of the total available long-term data base, it was salvageable. It should be kept in mind that evaluators do not have a responsibility for salvaging deficient or defective data bases. However, sufficient analysis must be done to support a rational judgment regarding the rejection of or conditional use of a study. In such instances, the opinion of the OPTS pathologist should be obtained before a final decision is made.

Another important pattern of neoplasia is the one in which there are "consistently positive results in two sexes and in several strains and species and higher incidences at higher doses".[1] It is generally agreed, at an international level, that this type of pattern is the best evidence of a positive oncogenic response obtainable with animal studies. Obviously, this pattern has major biological significance for determining the oncogenic potential of the test substance. If such a pattern is present in appropriately designed and conducted studies, the substance should be considered an oncogen for experimental animals and a suspect oncogen for humans.

These two patterns represent extremes, permitting early decisions on a study, not usually encountered by OPTS evaluators. More frequently encountered are tumor patterns requiring special attention during data analysis and evaluation. Among these patterns are: (1) the potential associations of endocrine tumors; (2) tumors of the hematopoietic system and; (3) patterns of tumors frequently associated with liver tumors. There are also patterns of increased or decreased tumor incidence for some tumor types which are associated with body weight differences between the treated groups and their concurrent controls, or which may be related to aging.

It was pointed out in major consideration #3 that special care must be taken when evaluating incidences for neoplasms of the hematopoietic system. Haseman[34] in investigating tumor incidence patterns in Fischer 344 rats in 25 National Toxicology Program (NTP) studies found that leukemia/lymphoma incidence decreases, in both sexes, were frequently associated with increased liver tumor incidences in the treated groups. A clear biological explanation for this association was not apparent.

Decrease in tumor incidence associated with lower body weight gains, restricted food consumption, or diet quality has been frequently reported. Conybeare[35] reported on a study using outbred Swiss mice which received two diets,

on an ad lib. or restricted basis each. Table 13 presents spontaneous tumor incidence differences between the two feeding regimens. In both sexes on both types of diet, dietary restriction was associated with slightly better survival up to 18 months (time of study termination) and with significant decreases in the incidence of neoplasms of all types. The excess tumor incidence in mice fed ad lib. was accounted for by an excess of both lung tumors and liver tumors. Conybeare discusses these findings in relation to the interpretation of tests for carcinogenicity. Examination of food consumption and body weight gain data is helpful in determining if this phenomenon is present in a particular study.

Hottendorf and Pachter[36] analyzed the NCI experience in oncogenesis testing. Table 14 presents the most commonly found tumors in 98 positive studies. The liver was not only the most common tumor site, but the mouse liver was a tumor site about twice as often as the rat liver. In bioassays where the liver of only one species is considered, the mouse liver was involved five times as often as the rat liver.

It is this pattern of liver involvement among the NCI bioassays, other oncogenicity studies, and the Conybeare study which causes pathologists and toxicologists to be concerned about the significance of hepatic neoplasms in

mouse studies [37, 38] Tomatis et al., while not ignoring mouse data have concluded: "It does not imply that the chemical which has been tested with negative results in one or more species should be automatically regarded as having a possible carcinogenic effect on man solely on the grounds that it induces liver tumors in the mouse."[39]

From the data reported by Haseman, Conybeare, Hottendorf and Pachter, and Tomatis, et al.[34, 35, 36, 39] and others[37, 38, 40] it should be obvious that while mouse liver (and lung) tumor patterns may be very simple to identify, it may be most difficult to evaluate their significance as far as a potential oncogenic effect in man is concerned. Part II. B. 6 of (2), in part, states the following relating to mouse liver tumors:

> These Guidelines take the position that the mouse-liver-only tumor response, when other conditions for a classification of "sufficient" evidence in animal studies are met, should be considered as "sufficient" evidence of carcinogenicity with the understanding that this classification could be changed to "limited" if warranted when a number of factors such as the following are observed: the occurrence of tumors only in the highest dose group and/or only at the end of the study; no substantial dose-related increase

> in the proportion of tumors that are malignant; the occurrence of tumors that are predominately benign, showing no evidence of metastases or invasion; no dose-related shortening of the time to the appearance of tumors; negative or inconclusive results from a spectrum of short-term tests for mutagenic activity; the occurrence of excess tumors only in a single sex.

When an increased incidence in mouse liver tumors is observed it is necessary to examine all other chemical and biological properties of the test substance in order to arrive at a final judgment.[40] Because mice appear to harbor a significant population of preexisting initiated or latent tumor cells,[40, 41] some investigators have suggested that the requirement for a mouse study may be an unnecessary redundancy when a valid rat study exists.[42]

5. Dose-response Relationships

The term "dose" can be ambiguous in that its precise meaning depends, in part, on the route of administration, the particular interest of an investigator, and the context in which it is used. In this section, regardless of the complexities of route of administration, absorption, distribution and excretion, dose means that stated quantity or concentration

of a substance to which a living organism is experimentally exposed. Although, the term "response" can be applied to either beneficial or injurious effects observed at a specific dose, emphasis here is placed on the latter type of responses from a multiple dose regimen.

Of all the observations which might be made with respect to any biological effect, the most fundamental one is that correlative relationship existing between the dose administered and the response or spectrum of responses that is obtained. In essence, this is the classical definition of the term "dose-response relationship." The concept expressed by this term is indispensable to the identification, evaluation and interpretation of most pharmacological and toxicological responses to chemicals. It is therefore important for an evaluator to understand the basic assumptions which underlie and support the concept.

The primary assumption is that a dose-response relationship is firmly based on knowledge or a defensible presumption that the response (effect) observed is a result of exposure to a known substance. Correlative assumptions are: there is a receptor site(s) with which a substance interacts to produce the response(s); the observed response(s) and degree of response are related to the concentration of the substance at the receptor site(s); and, the concentration at the site(s)

is related to the dose received. Therefore the biological concept of dose-response relationship includes the basic assumptions that (a) the observed response is a function of the concentration at a site, (b) the concentration at a site is a function of the dose, and (c) response and dose are causally related.[43]

The essential purpose of long-term animal studies is the detection of valid biological evidence of the toxic and/or oncogenic potential of the substance being investigated. Therefore, protocols should, in an appropriate way, maximize the sensitivity of the test without significantly altering the accuracy and interpretability of the biological data obtained. The dose regimen has an extremely important bearing on these two critical elements. In this regard, two controversial concepts (i.e., maximum tolerated dose (MTD) and lack of oncogenic thresholds) have had a significant influence on the selection of doses for long-term oncogenicity studies and on the interpretation of observed dose responses. The evaluator should be continually aware that this influence may have a high probability of interjecting unintended biases into a data base and the subsequent evaluation. The no oncogenic threshold concept may also have had an inhibitory influence on the scientific discussion and development of methods for assessment of oncogenic potency as well as the development and use of animal oncogen ranking or classification systems by regulatory agencies.

Part II. B.6. of Reference 2 discusses the dosage regimen for long-term animal studies and states in part:

> Long-term animal studies at or near the maximum tolerated dose level (MTD) are used to ensure an adequate power for the detection of carcinogenic activity. Negative long-term animal studies at exposure levels above the MTD or partial lifetime exposure at the MTD may not be acceptable because of toxicity, or if animal survival is so impaired that the sensitivity of the study is significantly reduced below that of a conventional chronic animal study at the MTD. Positive studies at levels above the MTD should be carefully reviewed to ensure that the responses are not due to factors which do not operate at exposure levels below the MTD. Evidence indicating that high dose testing produces tumor responses by indirect mechanisms that may be unrelated to effects at lower doses should be dealt with on an individual basis.

Historically, the concept of "maximum tolerated dose" (MTD) arose from long-term oncogenicity screening studies which employed very limited dosage regimens and relatively small numbers of animals. The intent of the studies, under

these limited conditions, was to maximize the likelihood of observing an oncogenic response by administering as high a dose of chemical as feasible. Little consideration was given to determining valid dose-response relationships; the major emphasis was to establish whether or not the chemical had oncogenic potential in a qualitative sense. To accomplish this, an extreme condition (i.e., a MTD) was routinely employed in these studies. Presently the MTD term has almost as many different connotations as there are individuals who use it.[44, 45, 46] Conscientious attempts to accommodate the concept in long term studies have frequently caused dose level adjustments in one or more animal groups and these have frequently introduced interpretational difficulties at the termination of the study.[79]

For these reasons and others discussed below, the characteristics of the highest dose to be administered in modern long-term animal tests are presently being reconsidered and more clearly defined by a concerned scientific community. An Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation has recommended that the following end points from subchronic studies be used in selecting chronic dose regimens for NTP long-term studies: a) organ specific and/or systemic pathology; b) body weight and organ weight data c) clinical laboratory measurements and; d) pharmacokinetic data.[47]

The developing consensus can be expressed thus. Ideally, dose selection for long-term oncogenicity studies should maximize the detection of potential oncogenic dose response relationships and facilitate the extrapolation of these to potential risks for other species including humans. Therefore, the largest administered dose should be at one which produces signs of minimal toxicity that do not compromise biological interpretability of the observed responses. For example, the upper dose should not: (a) alter survival in a significant manner due to effects other than tumor production; (b) cause a body weight decrement from the concurrent control values of greater than 10-12%; (c) exceed 5% of the total diet; (d) produce toxic, pharmacologic, or physiologic effects that will shorten duration of the study or otherwise vitiate the study results.[45]

Some of the reasons for this changing attitude toward MTD are presented here. The potential interpretive difficulties associated with oncogenic dose-response relationships in animal groups exhibiting excessive mortality and/or excessive body weight differences, when compared with their concurrent controls, have been discussed. It is also known that excessive stimulation or inhibition of glandular activity through normal mechanisms or abnormal pharmacological and physiological effects of excessive dosage can complicate evaluation and interpretation of oncogenic dose-responses.[48, 49, 50, 51]

What is not so obvious is the potential problems created by severe tissue/organ injury produced by excessive dosage levels in long-term oncogenicity studies.[52] Evidence indicates that 7-methylguanine and O6-methylguanine are incorporated into liver DNA following administration of acutely toxic doses of the hepatotoxins hydrazine, carbon tetrachloride, and ethanol in rats and mice[53] and the male Syrian golden hamster.[54] This suggests that aberrant methylation of DNA may be a response to severe toxic insult or damage to the rodent liver. If this effect is confirmed for other substances which induce neoplasms only at or near severely toxic doses, it will have a significant bearing on the selection of the dose regimen for long-term oncogenic studies and the assessment of oncogenic risks for humans.[40] It is also known that exaggerated doses can alter, in biologically significant ways, normal metabolic functions and pharmacodynamic parameters.[55]

Although it can be logically argued that responses observed at exaggerated dose levels (e.g., doses far in excess of levels experienced under real or potential exposure conditions) legitimately fall within the classical dose-response concept, there is a developing suspicion, based on growing scientific evidence, that such doses are interjecting biases of considerable importance into the already difficult task of evaluating animal oncogenic dose responses and the assessment of their relevance

to human risk.[46, 56, 57, 58] It has been suggested[46, 47, 55] that the MTD concept, or at least the term, be abandoned and that the scientific community rely instead on adverse signs that are biologically important, but less severe than gross tissue injury or destruction, in judging the adequacy of the highest dose administered in long term oncogenicity studies.

A statement as to the adequacy of the dose regimen used should appear in the evaluation document. The rationale for this opinion should be concisely stated and should include a brief presentation of the toxic manifestations observed at each dose level. Special notation of unusual findings (e.g., disease processes unrelated to compound administration, bladder or kidney stones) and the dose level or levels at which they were observed should also be made. If a NOAEL is present for toxic signs, it should be identified in the evaluation document.

The term "threshold" can be defined as that value at which a stimulus just produces a sensation, is just appreciable, or comes just within the limits of perception. In toxicology and pharmacology the concept of a threshold dose is accepted as applying to biological responses of nearly all chemical and some physical stimuli (e.g. one source defines 23 types of measurable biologic thresholds).[59] Generally, the

concept is understood by toxicologists to mean that there is a dose for nearly all chemical substances below which no response is discernible or detected in the organisms exposed to it. If this lack of response is exhibited by a reasonable number of test subjects, the dose is assumed to be a subthreshold dose. The no oncogenic threshold concept is contrary to the generally accepted biological threshold dose concept and requires special consideration because of its potential impediment to competent scientific evaluation of oncogenicity data bases and risk assessments.

Gehring and Blau[60] have presented succinct arguments for both sides of this and the MTD controversy. To paraphrase them might lessen their impact. For this reason and for the convenience of the reader, they are quoted here. The reader should consult the original paper for other important aspects of oncogenic dose responses and the references cited therein.

Evidence Supporting a Threshold Concept is Substantial. Some of the Arguments Are:

1. Chemical carcinogenesis is a multistage process involving:
   a. Exposure, absorption, distribution, activation, deactivation, and elimination of the chemical per se or products formed from it.

b. Interaction with critical receptor sites leading to moleculary transmittable products.

c. Survival and proliferation of transformed cells to clinical cancer.

Interference with any of these processes may constitute a threshold. For example, there is a plethora of data showing that promoters, which in themselves cannot initiate cancer, can enhance greatly the incidence of cancer induced by administration of an initiator. Also, the damaged receptor site may undergo repair.

2. Alteration of the physiological status may either augment or inhibit the response to a carcinogen. For example, age, sex, nutrition, population density, hormonal state, or concomitant disease may affect the response to a carcinogen. This suggests that a precancerous status may exist or may be induced without development of cancer until the precancerous status attains some critical level or until the precancerous status can no longer be held in check by suppressive mechanisms, whatever they may be.

3. As the dose of a carcinogen is decreased, the latency period for cancer development increases. This phenomenon was revealed lucidly by Druckrey (1967), who noted that the dose multiplied by some power of time was constant, i.e., dt^n = constant in which n = 2 to 4. For all practical purposes, this relationship implies a threshold in that multiples of a lifetime will be required for expression of cancer in response to low doses. Albert and Altshuler (1973) utilized the increasing latency with decreasing dose of a carcinogen to formulate limits for unavoidable exposures to carcinogens.

4. Utilizing the relationship of dose to time-of-appearance of cancer, Jones and Grendon (1975) postulated that a number of cells in close proximity require transformation to allow development of an aberrant clone of cells and ultimately cancer. This multihit hypothesis, if true, will result in a marked reduction in the incidence of cancer as the dose is decreased for the same reason that trimolecular chemical reactions become negligible as the concentrations of the reactants are decreased.

5. For many chemical carcinogens, cancer occurs only when doses are given that exceed those needed to cause pathological responses, such as grossly and histologically discernible tissue damage. This is not surprising, since some cancers develop clinically in chronically inflamed or scarred tissue, e.g., colonic cancer in patients with ulcerative colitis or regional enteritis, squamous cell carcinomas in ulcers of burn scars, squamous carcinomas of the bladder in schistosomiasis, scar carcinomas in lung, carcinomas and sarcomas arising in osteocutaneous fistulas caused by chronic osteomyelitis, and carcinoma of the stomach in autoimmune (atrophic) gastritis (Laroye, 1974). Perhaps sarcomas induced locally by implants of inert solid material or local injections of chemical substances represent an experimental expression of these phenomena observed clinically. Even such substances as water, salt, glucose, and a host of other common nutrients are carcinogenic when given in this manner (Grasso and Golberg, 1966). Such evidence of carcinogenicity is discounted for the most part. Is it any less reasonable to discount similar evidence when the administered dose is transported to another site in the body where it causes chronic inflammation and subsequently carcinogenesis?

6. There is a substantial and growing body of evidence that carcinogenesis is subject to immuno-surveillance, particularly cell-mediated immunity (Roe and Tucker, 1974; Weisburger, 1975).

7. Stress, such as administration of unrealistically large doses of chemicals to laboratory animals, can enhance greatly the response to oncogenic viruses and perhaps other innate carcinogens as well. This has been demonstrated eloquently by Riley (1975) in C3H/He mice infected with Bittner oncogenic virus, the incidence at 400 days of age was 92% in those under stress and only 7% in those in a protected environment.

8. Man and animals live in a sea of potential carcinogens, most of which were not placed here by man. There is reasonable evidence, in both humans and animals, that over-nutrition, particularly excess dietary fat, is a major cause of cancer (Wynder, 1976; Weisburger, 1976). Malonaldehyde, a product of peroxidative fat metabolism which is also formed spontaneously in tissues, particularly when the diet is

> deficient in antioxidants, has been found to be carcinogenic (Shamberger et al., 1974). Selenium, an essential micronutrient, calcium (Krook et al., 1971), and egg whites and yolks (Szepsenwol, 1963) have all been reported to cause cancer when given in excess to experimental animals.
>
> Thus, it seems that excesses of many substances may be expected to induce cancer. Is it not reasonable to believe that below some threshold, these naturally occurring environmental carcinogens will exert no carcinogenic effect? The logical alternative is to believe that most any substance, including food, continually gives rise to small numbers of aberrant cells which eventually cause cancer if competing causes of death do not prevail. Adherence to the latter logic allows acceptance of exposure to levels of man-made chemicals which do not add measurably to the background flora of carcinogens, which is likely to be substantial although not well elucidated.

The authors[60] continue:

> Arguments that there is no threshold for chemical carcinogenesis are equally substantive. The principal argument is, in essence, that cancer is an expression of a permanent, replicable defect

resulting from amplification of a defect initiated in one cell by reaction of the chemical with a critical receptor. Once such a defect occurs in a cell, the cell may be dormant for years before expressing a discernible untoward effect. Unlike classical toxicological responses, division of a large dose of some carcinogens into smaller repeated daily doses does not abolish the response. Indeed, for dimethylaminoazobenzene, 4-dimethylaminostilbene, and diethylnitrosamine, the total cumulative dose necessary for carcinogenesis with small daily doses is smaller than the single dose required to produce an equivalent response (Druckrey, 1976; Schramel, 1975; Weisburger, 1975). However, it should be noted that the size of the repeated doses can be reduced further, resulting first in an increased latency for development and, finally, no experimentally discernible response. It is important to emphasize that these data were obtained on highly potent, direct-acting carcinogens. As the doses of such agents are increased, a less than linear increase in tumors should be anticipated because their innate reactivity will preclude proportionate increases in the active agent at the receptor site.

Another frequently referred to piece of evidence is that exhaustive experiments on radiation-induced cancer have not revealed a threshold within the realm of statistical

reliability. However, the validity of equating chemical carcinogenesis to radiation-induced carcinogenesis is questionable. Entry of radiation into a cell and release of its energy, leading presumably to the local generation of free radicals, is governed by physical chemical laws; hence, a particle of radiation is just as likely to do its dirty deed within the nucleus of a cell as elsewhere. Such is not the case for chemical carcinogens; all sorts of deactivating events are feasible and, indeed, likely to occur before the chemical reaches the critical receptor.

Thus the argument concerning what may occur on the low end of the dose-response curve continues. Until recently, the conflict did not have a major impact because of the philosophy of 'no threshold' was applied to only a few agents which were very potent carcinogens; and somewhat more generally to intentional food additives because of the Delaney Clause. However, the impact is developing rapidly into a galloping crisis because the philosophy of 'no threshold' is being extended to proclaim 'no safe level of exposure' to any chemical shown to be carcinogenic regardless of the dose of the chemical needed to elicit a discernible carcinogenic response. Not unexpectedly, the chemicals thought heretofore to be safe do increase cancer when huge doses are administered. In many cases,

the doses used have exceeded those required to cause marked toxicological effects.[60]

No matter how important or desirable the concepts of MTD and no oncogenic threshold may appear for prudent regulatory decisions, it must be kept firmly in mind that presently it is not known if a substance exhibiting an oncogenic response as a result of large doses represents a risk when only small exposure levels are encountered by humans. To treat the no oncogenic threshold concept as a proven scientific fact for all substances exhibiting oncogenic potential is contrary to a growing body of evidence that thresholds may in fact exist for some such substances,[52, 61] and it may result in the evaluator misinterpreting or overlooking important biological evidence contained in the data base or in auxiliary studies. The ED_{01} study has shown that in individual cases, complete carcinogens may show thresholds of a real or practical nature. In other cases they may not.[61] If in the evaluator's opinion the data indicates the possibility of a potential threshold effect, this should be stated and a rationale given.

Table 15 presents four oncogenic incidence patterns actually encountered in rodent long-term studies. Except for Figure D, an inhalation study, the route of compound administration was dietary. Since the data for each figure was selected to illustrate types of incidence patterns, i.e.,

without regard to tumor types and site or toxic, pharmacologic, or other influences which may have been present, the data sources and substances are not identified. The fact that all but one of the selected patterns were evidenced by female mice is purely accidental.

Figure A represents an incidence pattern which is generally thought of as the "classical" carcinogenic multidose response relationship. A malignant tumor, lung adenocarcinoma, incidence increases with each increase in dose increment and in such a manner that statistical conformation of the probable reality of a positive dose response relationship is hardly needed.

Figure B represents data which is in stark contrast to the data represented by Figure A and is of a more common occurrence. The incidence data exhibits a random pattern, statistical analysis does not produce even a borderline value, and all incidences fall within the expected incidence values (i.e., historical controls) for the mouse strain used. In this example, even if strenuous statistical efforts had produced a borderline p value for the high dose group, there is no evidence of a dose response relationship and the response has no <u>biological</u> relationship to treatment. (see Task Force situation 1). It should be kept in mind that a dose response relationship should be firmly based on knowledge or a defensible presumption that the observed response is causally

related to the dose. If these conditions are not met the reality of such a relationship may be illusory. Figures A and B represent extremes encountered by evaluators. In both of these cases, all that is required, other aspects of the study being adequate for evaluation and interpretation, is a competently performed verification of the data base and the application of the major oncogenicity considerations.

Unfortunately, the potential incidence patterns which may be encountered in long-term rodent studies are legion and often times require considerable analytical skill to identify a valid dose response relationship, or the lack thereof, for any particular tumor type or group of tumors. Figures C and D are only two varieties of the potential problem patterns encountered between these extremes.

Figure C presents incidence data which are frequently encountered for many tumor types. The doubling of the concurrent control incidence at the low and middle dose levels, on the surface at least, appears to identify a significant dose response relationship of biological importance and the tumor incidence at the high dose appears to be an artifact. While this evaluation and interpretation is tempting, it can be misleading unless the artifactual nature of the highest dose data can be identified. There is nothing in the biological dose response concept which requires multiple dose regimen relationships to be unidirectional. Therefore, this type of

pattern and its variations must be viewed as a real dose response in the absence of knowledge or a defensible presumption to the contrary. Such relationships may need considerable analysis before their biological significance can be interpreted.

Before attempting any complex statistical analysis, the reviewer should examine the biological data base for a defensible explanation of the incidence pattern. When acceptable historical tumor incidence data for the particular neoplasm is available, it should be compared with the observed incidence for each treated group and the concurrent control group. If all the study incidences are within the historical tumor incidence range and the expected time of tumor appearance, the study incidence data may not represent a treatment relationship and the situation is equivalent to the Task Force's situations 1 or 2. However, before this explanation is completely accepted, further analysis of the data base, including auxiliary data, for corroborative evidence should be performed and a defensible presumption for its acceptance presented.

Assuming that the situation just discussed does not pertain and that the biological data base appears to support the validity of the data represented in Figure C, the evaluator should try to discover an explanation for the 4000 ppm incidence. Significant trends and p values derived for the lower dose incidences do not prove that the 4000 ppm incidence is arti-

factual in nature. It is possible that the high level group value was heavily influenced by an incidental disease process exacerbated by severe toxicologic stress, or the 4000 ppm level was so toxic that it caused significant early mortality thus reducing the number of animals at oncogenic risk when compared to control and the other two treatment group survival/ mortality data. The high level may not have caused significant mortality but caused a severe reduction in body weight by some mechanism which resulted in the low tumor incidence. It is also possible that the 4000 ppm dose caused a biologically significant shift in metabolic pathways or distribution and elimination patterns.[58] Examination of existing metabolism and or pharmacodynamic data might be helpful in evaluating this possibility. The reader may recall a similar incidence pattern and the amount of analytical effort needed to interpret the results.[79] Even after this effort, the interpretation of the data remained speculative. If no scientifically defensible explanation can be identified for the response, this fact should appear in the review.

Figure D represents a tumor incidence pattern encountered in some long-term rodent studies, although not very frequently in such a dramatic form. Usually the tumor incidences for the control and lower dose levels are higher for most tumor types than the incidences presented in this example. Since, in this case, the data immediately suggests that a threshold

dose has been exceeded, between 5.6 and 14.3 ppm, the first place to look for a defensible explanation would be any available metabolic and pharmacodynamic data.[58] If this type of data does not provide reasonable corroberative evidence for this presumption, the evaluator should proceed, as suggested in the discussion of Figure C. In this case special attention to the concurrent control group may be of importance. Occasionally the concurrent control group data, male, female, or both, do not fall within the "expected normal range" for the particular strain or species even in the same laboratory. This phenomenon, sometimes called the "control effect" can be very troublesome in the interpretive process. In this case an experienced toxicologist will spend as much time, or nearly as much time, examining and evaluating the control data as he or she will in examining data from the groups receiving the various dose levels (i.e., the treatment groups). It must be kept in mind that the term "treatment" can have a specific meaning, as used in this part, or a generic meaning. In the latter sense, it connotes all the environmental influences, controlled and uncontrolled, which are inherent in any animal experiment. Sometimes the uncontrolled influences (e.g., diseases,) cause the control group or groups to exhibit aberrant data bases which may artificially produce statistically significant differences and false dose response relationships.

Because the biological reality of oncogenic dose-response relationships is so important to risk identification and assessment, it is reiterated that such relationships should be based on knowledge or a defensible presumption that the response and dose are causally related. The knowledge or presumption must be based on the biological, toxicological, metabolic, pharmacodynamic, and other evidence (i.e., weight-of-evidence) contained in the submitted documentation. The evaluator must strenuously resist the temptation to accept a P value(s) as the sole designator of a biological dose-response relationship or the sole determinant of an oncogenic effect. The reader should examine Misconceptions Regarding Significance and P[89] to maintain a balanced perspective regarding this matter. Evaluators must understand that the weight given to the level of statistical significance, (i.e., P value) is not an automatic consequence of some natural law, it is a scientific judgment. After careful consideration of the data, if an evaluator chooses to dismiss a statistically significant difference between a treatment group and the concurrent control group, the rationale should be succinctly presented.

6. Decrease in latency (time to tumor discovery) of neoplasms that are usually observed.

A latent period is generally understood to mean the interval between the application of a stimulus and the observation of a response.

Some chemicals are known to induce tumors in experimental animals at very high incidences, much earlier than is usually expected (precocity), and are for this reason sometimes used as positive controls (Table 16). As may be seen, 40-51 ppm diethylnitrosamine, orally, produced a 100% incidence of liver tumors in 20-35 weeks in two different rat strains. In the case of 7,12-dimethylbenz(a)anthracene intubation of 15-20 mg produced a 92-100% incidence of rat mammary tumors in females in 12-16 weeks and by skin painting in the mouse, 75 mg produced skin neoplasia in 10-25 weeks.

Without adequate serial sacrifices, which are rarely performed because of cost, tumor latency can only be derived accurately in the case of visible tumors such as those of the skin or mammary glands, or the rare tumor types that rapidly kill the test animal. Therefore, this major consideration may be less useful for evaluating the oncogenicity of many substances than other criteria. However, since some chemicals do shorten the latent period, it is prudent for the evaluator to perform whatever appropriate analysis the data may allow and make a statement concerning this potential effect. The statement might be nothing more than that the study data do not allow a defensible scientific assessment concerning the latent period to be made or that the analysis does or does not <u>suggest</u> a precocity of tumor development.

Careful examination of early deaths, those which occur during the first 15 months of a study, may provide some evidence of precocious tumor development, but unless the cause of death can be determined to be directly related to neoplasia [not easily done in most cases[23]], early death may produce a false impression of decreased latency. This is particularly true when one or more dosage level groups exhibit a differential survival rate which results in significant inequalities between a treatment group survival rate and its concurrent control group survival rate. An example of this pitfall has already been cited.[79]

Historical control incidences and time to tumor discovery data may be of aid in evaluating latency (see major consideration #1). These types of data together with analysis of elapsed time from study initiation to tumor discovery sometimes allows a qualified statement concerning a latent period to be made.[88] Sometime clinical observations such as those that relate to palpable tumors or which may be associated with neoplastic development such as hematuria, abdominal distention, or impaired respiration might be useful in defining the time a tumor was first suspected of being present. If the data allow, it is also sometimes useful to determine the total cumulative dose and the absolute amount in a single-dose received by tumor bearing individuals. This approach is based on the observation by Druckery that in most cases the

total tumor yield in any given organ or tissue is generally proportional to the total cumulative dose received, but the rate of tumor appearance (latency) is related to the absolute amount in an individual dose.[63] This is a time consuming approach and should be used only if there is substantial reason to be suspicious that a precocity of tumor development is likely to be present.

E. Auxiliary Evidence

If the animal data base gives clear evidence that tumors are induced at multiple sites in rats, mice, and/or other species and the tumors are not among those having a high spontaneous incidence, the problem of assessing the oncogenic potential of a chemical is diminished and auxiliary evidence may play a minor supporting role in the evaluation. Seldom, however, is this the case and evaluators must oftentimes deal with results which are confined to a single rodent species, or sex, or a tumor type which has a high background incidence (e.g., mouse lung and liver tumors). In such situations, auxiliary evidence regarding other toxic manifestations; metabolic pathways, genotoxicity, biochemical reactivity; and patterns of absorption, distribution, and elimination may play a critical role in the weight of evidence approach.[40] However, not all of this type of evidence need be given equal weight and the evaluator should apply prudent judgment, on a case-by-case basis, when deciding the strength of auxiliary evidence and its contribution to the evaluative process.

1. Mutagenicity Data

The current use of mutagenicity data as an indication that a substance may have an oncogenic potential is based on the hypothesis that an alteration of genetic material in the affected cells is related directly or indirectly to tumorigenesis. This

process is thought to proceed by a series of events. The first step, initiation, involves damage to DNA resulting in changes in heritable genetic information. Proliferation of the permanently altered (initiated) cells is thought to result in clone formation in the tissue of exposed individuals. The progression of the altered cells to benign or malignant tumors is thought to be dependent on a series of not well understood mechanisms. Short-term mutagenicity tests exploit the fact that many oncogenic substances have the ability to produce DNA damage or chromosomal anomalies.

It must be kept in mind that tumorigenesis may not always proceed in this multi-step manner and that some oncogens may be effective through mechanisms that do not cause genetic effects [i.e., do not damage DNA.[64]] In this regard IARC states:

> In view of the limitations of current knowledge about mechanisms of carcinogenesis, certain cautions should be emphasized: (i) at present, these [mutagenicity] tests should not be used by themselves to conclude whether or not an agent is carcinogenic: (ii) even when positive results are obtained in one or more of these tests, it is not clear that they can be used reliably to predict the relative potencies of compounds as carcinogens in intact animals; (iii) since the currently available tests do not detect all classes of agents that are active in the carcinogenic process

(i.e., hormones, promoters), one must be cautious in utilizing these tests as the sole criteron for setting priorities in carcinogenesis research and in selecting compounds for animal bioassays.[18]

Furthermore, an international commission[65] recently evaluated the usefulness of mutagenicity studies as an approach to oncogenesis and concluded:

a) Genotoxic tests for chemical carcinogens are a product of research conducted during the past 10 years. The research efforts into these tests are still progressing rapidly. Therefore it should be anticipated that presently available test systems may be superceded by new tests with greater predictive value.

b) The use of an individual test or battery of present tests for genotoxicity as predictors for the carcinogenicity of specific chemicals does not give absolutely accurate results. <u>These tests should therefore be supplemented by carcinogenesis bioassays in animals</u> if specific chemicals are expected to enter the environment in appreciable quantities. The genotoxicity tests are of use (1) in selecting chemicals under development for possible adverse genetic or carcinogenic effects before costly product development is attempted; (2) in screening pres-

ently available natural or synthetic chemicals for genotoxic or carcinogenic potential; (3) in screening human body fluids or excreta for genotoxic agents that may indicate exposure to noxious agents; and (4) in understanding the mechanisms of cancer or mutation induction.

(c) Knowledge of the basic mechanisms of carcinogensis in animals is still in a primitive state. This subject needs increased research if the present hypothesis, based on correlative evidence that genotoxic mechanisms are involved in carcinogensis is to be accepted. Experimental evidence that mutagenicity is indeed part of the carcinogenic process would greatly increase confidence in the validity of the tests discussed in this report.[65]

Heedful of the cautionary statements, mutagenicity data used in conjunction with long-term rodent studies, can be useful in evaluation of oncogenic hazards since they appear to be able to separate, in some cases, those substances which are genotoxic (i.e., react with genetic materials) from those substances which do not appear to do so.

Wright considers genotoxic agents under two main headings: Precursors Agents - possessing no genetic properties per se but are converted into ultimate genotoxic agents by metabolism in

susceptible organisms; and Ultimate Agents - possessing the intrinsic properties necessary for interaction with critical cellular targets, e.g., alkyating agents, thereby initiating the genotoxic process.[66]

2. Metabolic - Pharmacodynamic Data

This rubric covers any data which may be concerned with the complex of physical and chemical processes involved in the functioning of any specific substance in, or its actions on, living systems. It is therefore very broad in scope and the reader must rely on the cited references for more detailed discussions of the technical aspects of this type of auxiliary evidence. Other aids available to the evaluator include (1) the Office of Pesticide Program Standard Evaluation Procedure (SEP) - Reviewing Metabolism Studies, and (2) the Chemical Information System. The latter is a computerized collection of chemical and regulatory data bases that allow structure, substructure, and name searching of many thousands of unique substances. It can be used to obtain lists of structurally related chemicals and also allows searching of the NIH, EPA, NIOSH, Registry of Toxic Effects of Chemical Substances and other toxicology data bases to determine if all or some of the structurally related chemicals have a common toxicological property.

An understanding of the mammalian metabolism of a chemical agent is basic to the discovery of probable oncogenic mechanisms (see IARC definition) and an understanding of chemical toxicity in general.[67, 68, 69] A prudent investigator would start such studies prior to initiating long-term rodent studies because, in addition to identification of major metabolites and metabolic patterns, it is extremely useful to have information on the potential effects a long-term dose regimen may have on such entities and relationships.[55, 67]

Consideration of the structures of ultimate carcinogens has led to the important generalization that such agents are strong electrophiles, mainly alkylating and arylating agents, although some carcinogenic acylating agents are known. In certain cases the instability of the presumed ultimate carcinogen prevents chemical synthesis. In such instances, e.g., the 2,3-epoxide of aflatoxin B_1, the nature of the ultimate reactant has been inferred from the structures of adducts generated by reaction of the formed products with biomacromolecules in situ. There are a few apparent exceptions to the generalization that ultimate carcinogens are electrophilic reactants. One such exception, 6-mercaptopurine, has been reported to cause an increase of certain tumors in the haemopoietic system of rats and mice.[66]

For further discussion of biochemical reactivity in relationship to oncogenicity see References 70 and 71, and for its importance to chemical toxicity in general see References 72 and 73.

The usefulness of animal toxicity and oncogenicity data is also enhanced by knowledge of the absorption, distribution and elimination patterns of the test substance, i.e., application of pharmacodynamic principles. Discussions and examples of the integration of this type of data with chronic toxicity data and macromolecular events associated with toxicity are available (e.g., styrene, vinyl chloride, and dioxane as well as the implications of this type of data for risk estimation.[74, 75, 76, 77]

F. Completion of Anaylsis

At this point the evaluator should have formulated tentative judgments and supporting rationale concerning: a) the acceptability of the evidential documentation and data base; b) the presence or absence of biologically important toxic and/or oncogenic effects and the relevancy of any modifying factors; and c) the likelihood that any of the adverse effects were induced by the tested substance.

Prior to applying the criteria presented in Part II, an evaluator should summarize, briefly and cogently, the critical biological and auxiliary data together with any modifying factors for all studies under review. Any rationale pertinent to an evaluation of the oncogenic potential of the substance should also be included in the summary. The following outline is suggested It should be modified according to the constraints of the data base.

1. Acceptability of each study considered.
2. Toxic effects.
3. Increased incidence of one or more histogenetically different types of neoplasms in multiple a) species, b) strains, c) sexes, and d) doses.
4. Increased incidence of neoplasms in multiple experiments with consideration of different routes of administration and/or dosage levels and relationships).

5. Increased incidence of neoplasms to an unusual degree (with respect to type, site, latency, malignancy and quantitative considerations).
6. Auxiliary evidence.

II. Evaluation and Classification of Evidence of Oncogenic Potential from Animal Studies

As stated previously, the essential purpose of long-term animal studies is the detection of valid biological evidence of the toxic and/or oncogenic potential of the substance being investigated. Clayson et al. discuss four areas of particular difficulty in the interpretation of oncogenicity tests: a) the heterogeneous nature of carcinogens in terms of exerting their effects by a series of differing mechanisms; b) meaning of a negative animal bioassay; c) significance of tumors induced against a high spontaneous incidence; and d) transspecies extrapolation. The authors conclude that prevailing evidence clearly points to the fact that mechanistic considerations taken together with data on carcinogen potency, dose-response relationships, and general toxicity will, in the future, lead to an increased ability to refine risk estimates. Approaching the regulation of carcinogens within such a conceptual framework makes it possible to exercise scientific judgment regarding the magnitude of risk. This is essential if we are to base decisions on sound scientific principles.[78] This paper should be read by all reviewers involved in oncogenicity evaluations.

The strength or weight-of-evidence from animal studies as well as that of any available auxiliary evidence, should be evaluated and classified by some agreed upon criteria before

mathematical calculation of risk is attempted. Part IV. B. of Reference 2 presents the following guidance for weighing such evidence. These assessments are classified into five groups:

1. Sufficient evidence* of carcinogenicity, which indicates that there is an increased incidence of maligant tumors or combined maligant and benign tumors**: (a) In multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose-levels) or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.
2. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) The studies involve a single species, strain, or experiment; or (b) the experiments are restricted by inadequate dosage level, inadequate duration of exposure to the agent, inadequate period of follow-up, poor

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* Under specific circumstances, such as the production of neoplasms that occur with high spontaneous background incidence, the evidence may be decreased to "limited" if warranted (e.g., there are widely diverging scientific views regarding the validity of the mouse liver tumor as an indicator of potential human carcinogenicity when this is the only response observed, even in replicated experiments in the absence of short-term or other evidence).

** Benign and malignant tumors will be combined unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same morphologic type.

survival, too few animals, or inadequate reporting; or (c) an increase in the incidence of benign tumors only.

3. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

4. No evidence, which indicates that there is no increased incidence of neoplasms in at least two well-designed and well-conducted animal studies in different species.

5. No data, which indicates that data are not available.

The categories "sufficient evidence" and "limited evidence" refer only to the strength of the experimental evidence that these agent(s) are carcinogenic and not to the power of their carcinogenic action.

Part IV. C. of Reference 2 also contains guidance for weighing of the total evidence (human and animal data) in a stratified scheme as follows:

Group A - Human Carcinogen

This category is used only when there is sufficient evidence from epidemiologic studies to support a causal association between exposure to the agent(s) and cancer.

Group B - Probable Human Carcinogen

This category includes agents for which the evidence of human carcinogenicity from epidemiologic studies ranges from almost "sufficient" to "inadequate." To reflect this range, the category is divided into higher (Group B1) and lower (Group B2) degrees of evidence. Usually, category B1 is reserved for agents for which there is at least limited evidence of carcinogenicity to humans from epidemiologic studies. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard agents for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans. Therefore, agents for which there is inadequate evidence from human studies and sufficient evidence from animal studies would usually result in a classification of B2.

In some cases, the known chemical or physical properties of an agent and the results from short-term tests allow its transfer from Group B2 to B1.

Group C - Possible Human Carcinogen

This category is used for agents with limited evidence of carcinogenicity in animals in the absence

of human data. It includes a wide variety of evidence: (a) definitive malignant tumor response in a single well-conducted experiment, (b) marginal tumor response in studies having an inadequate design or reporting (c) benign but not malignant tumors with an agent showing no response in a variety of short-term tests for mutagenicity, and (d) marginal responses in a tissue known to have a high and variable background rate.

In some cases; the known physical or chemical properties of an agent and results from short-term tests allow a transfer from Group C to B2 or from Group D to C.

Group D - Not Classified

This category is used for agents(s) with inadequate animal evidence of carcinogenicity.

Group E - No Evidence of Carcinogenicity for Humans

This category is used for agent(s) that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both epidemiological and animal studies.

TABLE 1

INCIDENCE (PERCENT) OF FEMALE CONTROL RATS BEARING THYROID C-CELL TUMORS AMONG ANIMALS SACRIFICED POST 12-MONTHS**
(Same Lab)

STUDY*	ADENOMA or CARCINOMA	ADENOMA	CARCINOMA
1			
Group A	10/58 (17.2)	10/58 (17.2)	0/58 (0)
Group B	7/59 (11.9)	6/59 (10.2)	1/59 (2)
2			
Group A	5/59 (8.5)	5/59 (8.5)	0/59 (0)
Group B	6/58 (10.3)	6/58 (10.3)	0/58 (0)
3			
Group A	9/57 (15.8)	6/57 (10.5)	3/57 (5)
Group B	6/55 (10.9)	5/55 (9.0)	1/55 (2)
4			
Group A	2/58 (3.4)	2/58 (3.4)	0/58 (0)
Group B	0/55 (0.0)	0/55 (0.0)	0/55 (0)
TOTAL	45/459(9.8)	40/459(8.7)	5/459(1.1)

* Each listed study had two control groups, identified as Group A or B. The rat strain is Sprague-Dawley.

TABLE 2

HISTORICAL CONTROL INCIDENCE OF LUNG TUMORS IN MALE B6C3F$_1$ MICE RECEIVING CORN OIL BY GAVAGE**
(Different Labs)

LABORATORY	Alveolar/ Bronchiolar Adenoma	Alveolar/ Bronchiolar Carcinoma	Alveolar/ Bronchiolar Ademoma or Carinoma
A	8/100 (8.0%)	6/100 (6.0%)	14/100 (14.0%)
B	12/235 (5.1%)	17/235 (7.2%)	29/235 (12.3%)
C	5/120 (4.2%)	3/120 (2.5%)	8/120 (6.7%)
D	19/150 (12.7%)	4/150 (2.7%)	22/150 (14.7%)
E	4/49 (8.2%)	3/49 (6.1%)	7/49 (14.3)
F	32/248 (12.9%)	11/248 (4.4%)	43/248 (17.3%)
TOTAL	80/902 (8.9%)	44/902 (4.9%)	123/902 (13.6%)

** Nota Bene - This data is for illustrative purposes only. It must not be used for any other purpose.

TABLE 3

EXAMPLES OF NCI USE OF HISTORICAL CONTROL DATA*

	EXAMPLE I	EXAMPLE II
REFERENCE:	(TR-160)	(TR-145)
LESION:	Hepatocellular Carcinoma	Endometrial Polyps
SEX/SPECIES:	Male $B6C3F_1$ Mice	Female Fisher 344 rats
TUMOR RATES:	Controls: 5/20, 25% Low-Dose: 26/50, 52% High-Dose: 27/50, 54%	Controls: 0/19, 0% Low-Dose: 4/50, 8% High-Dose: 9/50, 18%
SIGNIFICANT:	Trend: P = 0.039 Low-Dose: P = 0.035 High-Dose: P = 0.025	Trend: P = 0.018 Low-Dose: NS High-Dose: P = 0.044 (borderline)
INTERPRETATION:	Neoplasm not related to treatment	Polyps not related to treatment
COMMENT:	Historical control rate 137/422(32%) range up to 58% compared to 5/20 (25%) in study control group	Historical control rate 28/284(10%) compared to 0/19(0%) in study control group

* NB: These examples are for illustrative purposes only. Consult references (12) and (13) for full data base.

TABLE 4*

SUMMARY OF SPONTANEOUS TUMORS OBSERVED UPON RE-EXAMINATION OF SERIAL SECTIONS OF SELECTED TISSUES FROM 177 (63 MALES, 114 FEMALES) SPRAGUE-DAWLEY RATS

Type of tissue and tumor	No. of organs	No. of tumors				Age in days
		Single section vs. serial section				
		Male	Female	Male	Female	
Thyriod	140					
light cell adenoma		4	5	24	31	300-960
Adrenal	143					
pheochromocytoma		5	2	7	4	540-930
cortical carcinoma		1	0	1	0	690
Hypophysis	50					
adenoma		3	2	4	4	360-900
Ovary	61					
granulosa cell tumor		-	1	-	1	600
papillary adenocarcinoma		-	0	-	1	660
Uterus	51					
leiomyosarcoma		-	2	-	2	480-720
endometrial polyp		-	1	-	6	420-690
Brain	126					
ependymoma		1	0	1	0	330
papilloma, choriod plexus		0	1	0	1	660
meningioma		0	1	0	1	510
pinealoma		1	0	1	0	480
Testes	45	0	-	0	-	-
Totals		15	15	38	51	

*From reference (14) Table 1, p. 834.

Table 5*

Tumors and organs of origin in 2,082 rats of 6 sources-Continued

Tumors	Sprague-Dawley	Holtz-man-SD	Charles River-SD	Diablo-SD	Osborne-Mendel	Oregon	Total
Number of rats	258	268	535	217	131	673	2,082
Brain:							
Glioma	2	2	3	3	1	4	15
Ependymoma	.....	.....	.....	1	.....	1	2

*Selected form reference (15) Table 2, pp. 1245-46.

TABLE 6*

PROLIFERATIVE CHANGES AND
THEIR SYNOMYMS IN RAT LIVER

CHANGES	SYNONYMS
Hyperplasia (Not Neoplasm)..... :	Foci and areas of cellular alteration (clear cell, basophilic-, acidophilic-Vacuolted-) Hyperplastic foci and areas Basophilic hyperplasia Hyperplastic nodule Nodular hyperplasia
Benign Neoplasm..... :	Adenoma Neoplastic nodule Hyperplastic nodule Nodular hyperplasia Hepatoma Hepatic cell adenoma Trabecular carcinoma
Malignant Neoplasm..... :	Hepatocellular carcinoma Hepatic cell carcinoma Hepatoma Hepatoma Type 1 Hepatoma Type II Hepatoma malignant Liver cell carcinoma

*From ref. (18)

Table 7*

NEOPLASMS (TUMORS, NEW GROWTHS)

ONE OF THE DEFINITIONS: NEOPLASM IS AN UNCONTROLLED GROWTH OF CELLS

SOME CHARACTERISTICS

BENIGN NEOPLASMS

GROSS CHANGES:

1. Slow growth
2. Expansive type of growth
3. May be capsulated
4. Well defined contoure
5. Focal appearance
6. Not ulcerated
7. Usually not necrotic

HISTOPATHOLOGIC CHANGES:

1. Less anaplastic
2. Not metastatic or infiltrative
3. Moderate cellularity
4. Nuclear chromatin resembles normal
5. Moderate structural differences from normal tissues
6. Low mitotic index
7. Moderate change in nucleus and cytoplasm ratio

MALIGNANT NEOPLASMS

GROSS CHANGES

1. Fast growing
2. Infiltrative or metastatic growth
3. Not capsulated
4. Undefined contoures
5. Diffuse or systematic appearance
6. May be ulcerated
7. May be necrotic

HISTOPATHOLOGIC CHANGES:

1. Highly anaplastic
2. Metastatic or infiltrative
3. Marked cellularity
4. Hyperchromatic nuclei
5. Marked structural differences from normal tissues
6. Increased mitotic index
7. Marked change in nucleus and cytoplasmic ratio

*From ref. (1%)

Table 8*

Potential differences between chemcially-induced and tumors in control rodents

	Tumors in control animals	Induced tumors
Histogenesis		
Hyperplasia	not evident	present
Preneoplastic lesions	not readily evident	present
Precancerous lesions	not evident	present
Morphology		
General	characteristic of tissue for strain of rodent	sometimes different from usual control tumor
Histologic tumor types	one type	several types
Stromal lymphoid respones	usually absent	may be present
Biologic behavior	often benign	more often malignant
Multiplicity	singular	multiple, often involves entire organ or tissue

*Ref. (19) Table 2 p. 282

TABLE 9

LIVER (Animal No.)																							
Hepatocellular Carcinoma																							P
Hepatocellular Adenoma (No. Present)	1	6	2	5	1	4	5		4		1	5		2	7	1	N	1	1		4	4	
Malignant Lymphomas																							
Granulocytic Leukemic																							
Angiosarcoma																							
Carcinoma, Metastatic																							
Sarcoma, Metastatic																							
Reticulum Cell Sarcoma																							
Hepatocholangiocarcinoma																							
Multifocal Hepatocellular Degeneration																							
Basophilic Foci			2																				
Mononuclear Cell Infiltration	1					2				3		1	1		2	1				1			
Foci of Mononuclear Cells																							
Angiectasis						2							3										
Focus of Cellular Change														3									
Multifocal Hepatitis	2	2	4		3	3	3	1		3		2	2	2		3			2			4	
Multifocal Necrosis												2										2	

Key: P = Present N = No Section A = Autolysis X = Not remakable
1 = Minimal 2 = Slight 3 = Moderate 4 = Moderately Severe High
5 = Severe/High 1 = Incomplete Section

TABLE 10

	GROUP I			GROUP II			GROUP III			GROUP IV		
	Scheduled Sacrifice	Moribund Sacrifice & Deaths	Total	Scheduled Sacrifice	Moribund Sacrifice & Deaths	Total	Scheduled Sacrifice	Moribund Sacrifice & Deaths	Total	Scheduled Sacrifice	Moribund Sacrifice & Death	Total
LIVER (NO. EXAMINED)	(22)	(52)	(74)	(34)	(42)	(76)	(24)	(52)	(76)	(22)	(53)	(75)
Hepatocellular Carcinoma		4	4	2	1	3	2	1	3	1	1	2
Hepatocellular Adenoma*	2/2	1/1	3/3	1/1	4/3	5/4	30/12	24/11	54/23	54/17	21/12	75/29
Malignant Lymphoma	3	9	12	2	7	9	1	7	8		6	6
Granulocytic Leukemia		1	1								1	1
Angiosarcoma								2	2		1	1
Carcinoma, Metastatic											1	1
Sarcoma, Metastatic		1	1		1	1						
Reticulum Cell Sarcoma				1		1		1	1			
Hepatocholangio-carcinoma												
Multifocal Hepatocellular												
Degeneration		1	1				1	2	3		3	3
Basophilic Foci				1		1				1	2	3
Mononuclear Cell Infiltration	6	9	15	9	1	10	9	2	11	8	8	16
Foci of Mononu-clear Cells				1		1					1	1
Angiectasis	1		1	2	1	3		1	1	2	3	5
Focus of Cellular Change				3		3				1		1
Multifocal Hepatitis	16	10	26	23	4	27	13	7	20	14	9	23
Multifocal Necrosis	2	6	8	3	8	11	1	6	7	2	6	8

* Number of neoplasms/number of animals with neoplasms.

Table 11*

Guidelines for Combining Benign and Malignant Neoplasms

in the Fischer 344 Rat and B6C3F1 Mouse

Tissue	Tumors	Combine
Liver	Neoplastic nodule-rat or Hepatocellular adenoma-mouse Hepatocellular carcinoma	Yes
	Bile duct ademoma Bile duct carcinoma	Yes
Mammary Gland	Fibroma Fibroadenoma	Yes
	Carcinoma Adenocarcinoma	Yes
	Fibroma/Fibroadenoma Carcinoma/Adenocaricinoma	No
Thyroid	Follicular cell adenoma Follicular cell carcinoma	Yes
	C-cell adenoma C-cell carcinoma	Yes
	Follicular cell tumors C-cell tumors	No
Pituitary	Adenoma Carcinoma	Yes
Lung	Bronchioalveolar adenoma Bronchioalveolar carcinoma	Yes
Hematopoietic System	Rat Leukemia mononuclear cell (Fischer rat) Lymphocytic Undifferentiated	Yes
	Myelogenous Leukemia Leukemias-other types	No
	Malignant lymphoma (lyphosarcoma) Lymphocytic Lymphoblastic Histiocytic Reticulum Cell Mixed cell	Yes

*From ref. (20) pp 7-11

	Leukemias-all types Lymphomas-all types	No
	Mouse Lymphocytic Leukemia Undifferentiated Leukemia	Yes
	Myelogenous leukemia Leukemia-other types	No
	Malignant lymphoma (lymphosarcoma) Lymphocytic Lymphoblastic Histiocytic Reticulum cell	Yes
	Leukemias-all except myelogenous Lymphomas-all type	Yes
Pancreas	Islet cell adenoma Islet cell carcinoma	Yes
	Acinar cell adenoma Acinar cell carcinoma	Yes
	Islet cell tumors Acinar cell tumors	No
Gastrointestinal Tract	Forestomach papillomas Squamous cell carcinomas	Yes
	Glandular region and intestine Adenomas/Adenomatous polyps Adenocarcinomas	Yes
	Leiomyomas Leiomyosarcomas	Yes
	Fibromas Fibrosarcomas	Yes
	Squamous cell tumors Glandular tumor Mesenchymal tumor	No
	Leiomyomas/leiomyosarcomas Fibromas/fibrosarcomas	No
Kidney	Tubular cell adenoma Tubular cell carcinomas	Yes
	Transitional cell papillomas Transitional cell carcinomas	Yes

	Lipomas Liposarcomas	Yes
	Transitional cell tumors Tubular cell tumors	No
	Lipomatous tumors Other types of renal tumors	No
Urinary Bladder	Transitional cell papillomas Transitional cell carcinomas	Yes
Skeletal System	Osteoma Osteosarcoma	Yes
	Crondroma Chrondrosarcoma	Yes
	Osteoma/Osteosarcoma Chrondroma/Chrondrosarcoma	No
Adrenal Gland	Cortical adenomas Cortical carcinomas	Yes
	Pheochromocytoma Malignant pheochromocytoma	Yes
	Cortical tumors Medullary tumors	No
Brain	All gliomas, i.e. Oligodendroglioma Astrocytoma	Yes
	Granular cell tumors Gliomas	No
	Nerve cell tumors Gliomas	No
	Meningiomas-all types Other CNS tumors	No
Ovary/Testicle	Germ cell tumors-all types	Yes
	Stromal tumors-all types	Yes
	Germ Cell tumors Stromal tumors	No

Uterus	Stromal polyps Stromal sarcomas	Yes
	Glandular adenomas Adenocarcinomas	Yes
	Stromal tumors Glandular tumors	No
Integument	Basal cell tumors all types Pilomatrixoma	Yes
	Sebaceous gland tumors-all types	Yes
	Squamous cell papilloma Squamous cell carcinoma	Yes
	Squamous cell tumor Adexal tumors	No
	Basal cell tumors Squamous cell tumors	No
	Keratoacanthoma Squamous cell carcinoma	No
Subcutis	Fibromas Fibrosarcomas	Yes
	Hemangiomas Hemagiocarcomas	Yes
	Leiomyomas Leiomyosarcomas	Yes
	Fibromas/fibrosarcoma Lieomyomas/leiomyosarcomas	No
	Connective tissue tumors Endothial tumors	No
Preputial/Clitoral Gland	Adenoma Carcinoma	Yes
Zymbal Gland	Adenoma Carcinoma	Yes
Nasal Cavity	Adenoma Adenocarcinoma	Yes
	Squamous cell tumors Glandular tumors	No
	Esthestioneuralepithelia tumors Other tumors	No

TABLE 12

Total Number of Rats with Neoplasms (all types)

Male		Dosage Group	Females	
No.	%		No.	%
46/60	76.7	1*	49/60	81.7
38/58	65.5	II	48/60	80.0
37/57	64.9	III	47/59	79.7
38/58	67.2	IV	47/59	79.7

*Control

TABLE 13*

Incidence of Neoplasm in Mice Killed at End of Test

Dietary regime (sex)	No. of mice killed at end of test†	No. of mice with... Neoplasms at any site	Malignant neoplasms at any site	Lung tumours	Multiple lung tumours	Lung tumour of grade 2 or more§	Lung tumour of grade 3 or more§	Liver tumours	Multiple liver tumours	Liver tumour grade B, C or D¶	Liver tumour grade C or D¶	Neoplasms at sites other than lung or liver
PRD *ad lib.* (M)	48	19***	4	10	2	7	3	11***	4**	7*	3	3
PRD restricted (M)	55	8	1	6	0	4	1	2	0	2	0	1
41B *ad lib.* (M)	45	28***	4	13	1	10*	2	21****	7***	8	1	2
41B restricted (M)	51	16	1	10	3	5	0	6	0	5	0	1
PRD *ad lib.* (F)	48	12***	3	7**	0	5	0	2	0	0	0	4*
PRD restricted (F)	64	4	1	2	0	2	0	1	0	1	0	1
41B *ad lib.* (F)	51	14*	6	9	0	4	2	3*	0	2	1	5
41B restricted (F)	59	8	3	5	0	1	0	0	0	0	0	3
PRD *ad lib.* (M & F)	96	31****	7**	17**	2	12*	3	13***	4**	7	0	7**
PRD restricted (M & F)	119	12	2	8	(0)	6	1	3	0	3	0	2*
41B *ad lib.* (M & F)	96	42****	10*	22*	1	14**	2	24****	7***	10	2	7
41B restricted (M & F)	110	24	4	15	3	6	0	6	0	5	0	4
PRD/41B *ad lib.* (M)	93	47****	8**	23	3	17**	5	32****	11****	15**	1	5
PRD/41B restricted (M)	106	21	2	16	3	9	1	8	0	7	0	3
PRD/41B *ad lib.* (F)	99	26***	9*	16**	0	9**	2	5*	0	2	1	9*
PRD/41B restricted (F)	123	12	4	7	0	3	0	1	0	1	0	4
PRD/41B *ad lib.* (M & F)	192	73****	17***	39***	3	26***	7	37****	11****	17**	2	14**
PRD/41B restricted (M & F)	229	36	6	23	3	12	1	9	0	8	0	6
PRD *ad lib*/restricted (M & F)	215	43†††	9†	25	2	18	4	16†††	4	10	0	9
41B *ad lib*/restricted (M & F)	206	66	14	37	4	20	4	30	7	15	2	11

M = Male F = Female

†A total of 19 mice that died between weeks 80 and 83 have been excluded

§Lung tumours were graded as follows (based on Walters, 1966): 1 = benign non-invasive adenoma; 2 = adenoma (extending into airways and/or into surrounding lung); 3 = adenocarcinoma with metastases in lobe of origin or entirely replacing one lobe; 4 = adenocarcinoma extending through pleura or metastasizing to lobes other than the lobe of origin; 5 = adenocarcinoma metastasizing to sites outside the thorax

¶Liver tumours were graded as follows: A = consists of almost normal-looking liver cells in almost normal arrangement; B = consists of recognizable parenchymal cells arranged in cords; C = undoubtedly malignant liver cell tumour that has not metastasized elsewhere; D = metastasizing liver cell tumour.

Values marked with asterisks are significantly higher than the corresponding values given in the line below (*$P < 0.1$; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$); those marked with daggers are significantly lower than the corresponding values in the line below (†$P < 0.1$; †††$P < 0.01$)

*From (35) Table 3, p. 71

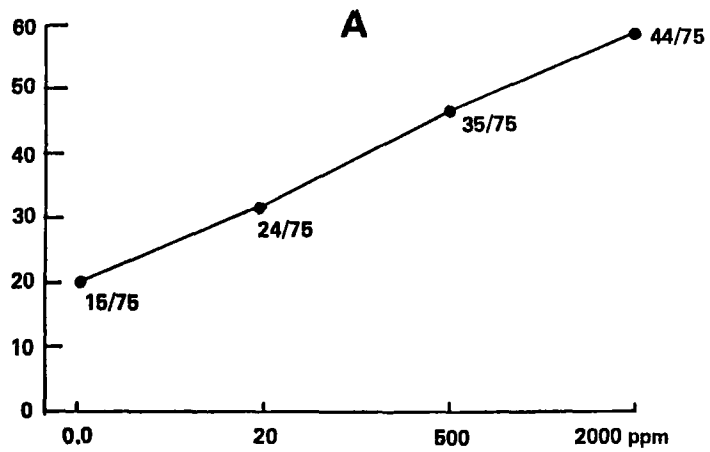
TABLE 14*

Most Commonly Induced Tumors in the 98 Positive NCI Bioassays

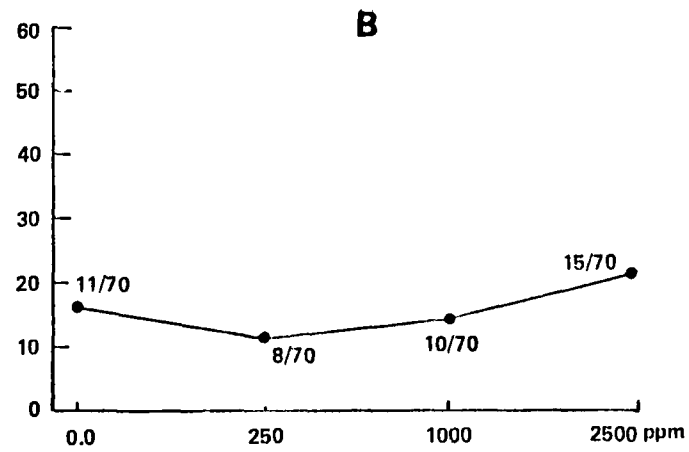
Site or Tumor Type	Rat		No. Rat Bioassay Involved	Mouse		No. Mouse Bioassays Involved	Total No. Bioassays Involved
	Male	Female		Male	Female		
Liver	18	15	21	31	44	50	55
Mammary Gland	1	13	13	0	3	3	16
Lymphoma/Leukemia	5	3	6	4	7	8	13
Lung	1	2	2	7	7	7	13
Urinary Bladder	6	10	11	2	2	2	11
Forestomach	7	5	7	5	5	5	11
Thyroid	6	5	6	4	4	4	11
Hemangiosarcoma	3	1	3	6	5	7	10
Uterus	-	7	7	-	3	3	10
Zymbal Gland	7	8	9	0	1	1	9

*Ref. (23) Table 2, p. 24

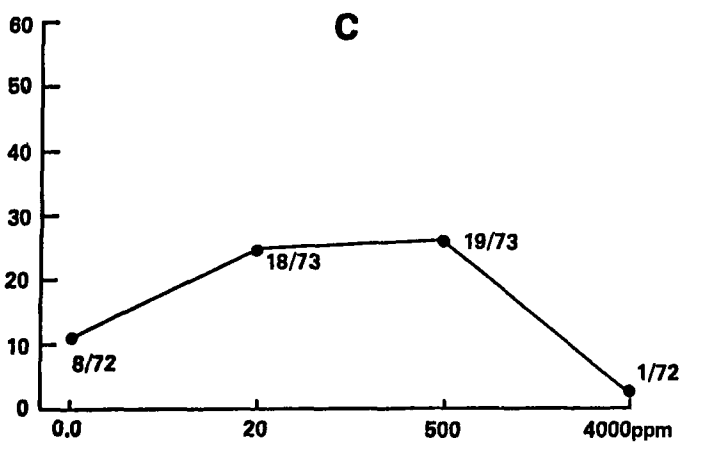
SELECTED TUMOR INCIDENCE PATTERNS



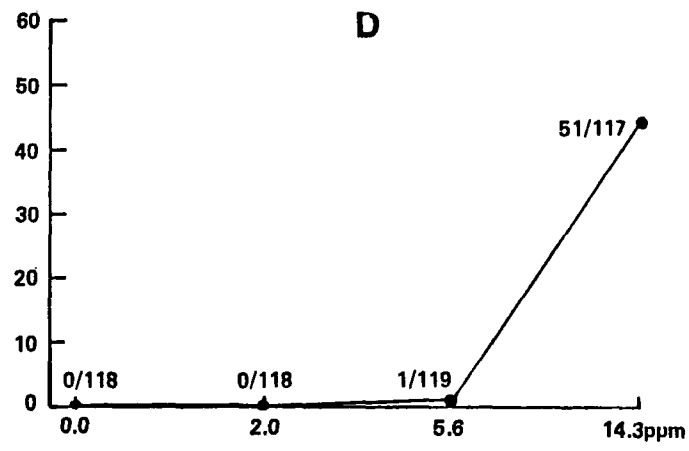
♀ CD -1 MICE Lung Adenocarcinoma



♀ SWISS MICE Lung Adenoma



♀ CD-1 MICE Reticulo-endothelial Tumors



FISHER 344 Rats Squamous-cell carcinoma

TABLE 16*

Typical Standard Carcinogens

Carcinogen	Species (strain)	Sex	Route	Dose	Main target Organ and incidence	Latent period (weeks)	References
Diethylnitrosamine	Rat (Fischer)	M or F	Oral	40 ppm (in water)	Liver 100%	20	13, 20
Diethylnitrosamine	Rat (CR-SD)	M or F	Oral	51 ppm (in water)	Liver 100%	35	17
N-2-Fluorenylacetamide	Rat (CR-SD)	M	Oral	223 ppm (in diet)	Liver 90%	26-40	17
		M	Oral	80 ppm (in diet)	Liver 30%	60-90	Unpublished
		F	Oral	80 ppm (in diet)	Breast 50%	60-90	Unpublished
N-2-Fluorenylacetamide	Rat (Fischer)	F	Oral	2mg (by gavage, 5 days per week)	Breast 20%	30-40	7
N-2-Fluorenylacetamide	Mouse	M or F	Oral	740 ppm	Liver	40	Unpublished
		M or F	Oral	240 ppm	Liver	90	Unpublished
Uracil mustard	Rat (Sprague-Dawley)	M	i.p.	11.5 mg/kg	Pancreas 4% Lymphoma 13% Peritoneum 22%	65 to death	6
		M	i.p.	23 mg/kg	Pancrease 8% Lymphoma 25% Peritoneum 33%	50 to death	6
		F	i.p.	11.5 mg/kg	Breast 55% Lung 10% Lymphoma 10% Peritoneum 10%	71	6
		F	i.p.	23 mg/kg	Breast 53% Lung 7% Lymphoma 14% Ovary 20% (0.5% controls) Peritoneum 40%	56	6

*From ref. (49) pp 31-33

Carcinogen	Species (strain)	Sex	Route	Dose	Main target organ and incidence	Latent period (weeks)	References
Uracil mustard	Mouse	M or F	i.p.	0.008 g/kg	Lung 100%	24	16
		M or F	i.p.	0.020 g/kg	Lung 100%	24	16
		M or F	i.p.	0.040 g/kg	Lung 100%	24	16
Uracil mustard	Mouse	M	i.p.	9.3 mg/kg	Lung 64%	61 to death	6
		M	i.p.	19.3 mg/kg	Lung 50%	45 to death	6
		F	i.p.	9.3 mg/kg	Lung 60% Ovary 25% Lymphoma 50%	58	6
		F	i.p.	19.3 mg/kg	Lung 60% Lymphoma 40% Ovary 33%	69	6
Urethane	Mouse (A/He)	M or F	i.p.	10 mg	Lung 100%	24	16
		M or F	i.p.	20 mg	Lung 100%	24	16
N,N-Demethyl-4-stilbenamine	Rat	M	Oral (in feed)	0.004%	Ear duct 63%	38	14a
3-Methyl-4-dimethyl-aminoazobenzene	Rat	M	Oral (in feed)	0.05%	Liver 55%	37	14a
Nitrogen mustard	Mouse (A/J)	M or F	i.p.	0.21 mg/kg	Lung 40%	39	15
		M or F	i.p.	0.87 mg/kg	Lung 69%	39	15
		M or F	i.p.	3.4 mg/kg	Lung 95%	39	15
7,12-Dimethalbenz(a)-anthracene	Rat (SD)	F	Oral	15-20 mg (by tube)	Breast 92-100%	12-16	19
7,12-Dimethylbenze(a)-anthracene	Mouse	M or F	Skin	75 mg	Skin	10-25	3
3-Aminotriazole	Rat	M or F	Oral	300 ppm	Thyroid		14
		M	Oral	300 ppm	Liver 65%		14
		F	Oral	300 ppm	Liver 48%		14

Carcinogen	Species (strain)	Sex	Route	Dose	Main target organ and incidence	Latent period (weeks)	Reference
3-Aminotriazole	Mouse (C57Bl/6XC3H/Anf)f_1	M or F	Oral	2,192 ppm	Thyroid Liver	78	10
Safrole	Rat (Osborne-Mendel)	M or F	Oral	5,000 ppm	Liver	104	11
	Mouse (C57Bl/6XC3H/Anf)f_1	M or F	Oral	1,112 ppm (in diet)	Liver	82	10

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NB. The following references are from an internal EPA document which contains confidential data. Removal of such data will eliminate the instructional values of the document. Therefore the document must not be released from HED without the proper authorization and legal clearances.

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